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REVIEW ARTICLE

# From wild to captive: Understanding the main nutritional diseases of sharks in public aquariums

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**ABSTRACT**

The establishment of a well-adapted and accurate feeding regimen is a fundamental determining factor in the maintenance of shark species in captivity. Replicating the natural diet in captivity is commonly accepted and recommended. Given the diverse nutritional composition of prey, supplementation may become necessary. The captive environment poses unique challenges, making sharks susceptible to an array of health issues, including numerous diseases. Mitigating these risks demands detailed husbandry practices, an appropriate physical environment and a balanced diet. Supplementation, encompassing vitamins and minerals, becomes imperative for the provision of essential nutrients. This complexity has rendered the formulation of an adapted feeding plan for aquarists exceptionally challenging. The scarcity of information in these species adds to the issue, mandating extrapolation from various shark groups and to species with analogous characteristics. This literature review concentrates predominantly on benthic and pelagic shark species prevalent in contemporary aquariums. The central argument posits that dietary choices in captivity rely on factors such as availability, quality and consistency of supply. The advocated approach highlights the importance of a balanced, diverse feeding that closely mirrors natural diets. It is then crucial to emphasize that these are general guidelines, and the specific dietary requisites may diverge between shark species. Collaborating with experts in marine biology, shark husbandry and veterinary care is imperative for the formulation and perpetuation of an adapted diet for captive sharks.

**KEYWORDS**

elasmobranchs, nutrition, public aquaria, foodborne diseases, marine predator

## INTRODUCTION

Sharks, being apex predators in various marine ecosystems, hold a significant ecological importance. However, they are also among the most endangered vertebrates on the planet. Zoos and public aquaria house a variety of elasmobranch species, yet there is a notable dearth of knowledge to effectively manage these captive populations and enhance their long-term viability (Janse et al., 2004a, 2004b). Regrettably, the care of such delicate species, particularly those obtained from the wild, presents challenges in terms of husbandry and behavioural

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considerations. Nutrition is one of the key processes to be understood in depth to keep a species in captivity, however, despite years of studying the feeding behaviour of sharks, little has been done in this field. Indeed, the basis of the health and survival of any animal in captivity is the access to a balanced feeding plan, as close as possible to the quality and quantity found in the wild. Together with an adequate environment, the food, vitamin and mineral supplementation, if correctly administrated, can provide a unique opportunity for aquaria to restore shark populations and open new research fields to understand these little known species essential to the food chain. Historically, elasmobranchs', and more precisely, shark nutritional requirements were entirely extrapolated from studies done in bony fishes (Janse, 2003). Understanding the basic anatomy of the digestive system of sharks, their metabolism and the importance of nutrients, are considered fundamental aspects of knowledge for describing the feeding plan they are most adapted to. These diet specifications are often not clearly defined in scientific literature, making it extremely difficult for aquarists to develop an appropriate feeding plan. Public aquaria often select their shark species based on hardiness (referring to the species' capacity to adapt and survive in captivity, also correlated to the size), availability (referring to the relative difficulty of collecting the animals from a given area) and compatibility (referring to the interaction compatibility between the shark species and the other animals kept in the same tank, and the compatibility with the type of display available) (Morris et al., 2010). It is very common to encounter the following species in public aquaria: the nurse shark (*Ginglymostoma cirratum*, Bonnaterre, 1788), the tawny nurse shark (*Nebrius ferrugineus*, Lesson, 1831), the zebra shark (*Stegostoma fasciatum*, Forster, 1781), or some pelagic shark species such as the grey reef shark (*Carcharhinus amblyrhynchos*, Bleeker, 1856) (Dehart, 2004). Even though those species show greater survival rate and adaptability in captivity, general illnesses do occur, often in relationship with the diet. Some of the most common ones include vitamin A imbalance, iodine deficiency or parasitic infection via the feed ingested (Hoopes, 2017). Exploring digestive enzyme roles in shark guts and their precise identification could illuminate vitamin and mineral requirements. Assuming sharks share nutrient needs with other vertebrates, extensive studies are lacking (Halver, 2002; Teles, 2012). Enzymatic activity techniques, common in other organisms, offer a potential avenue for exploring the molecular basis of dietary specialization in various shark species (German, 2011; Leigh et al., 2017).

This paper summarizes the main causes of food-borne health problems and gives an overview of the existing prevention and treatment methods. The compiled information serves as a foundational model for institutions, contributing to a deeper understanding of the captive care of shark species. The collected data can help improve feeding practices in captivity and provide suggestions to continue research in this critical field. These insights offer valuable suggestions to advance captive feeding practices, research and conservation efforts for these endangered marine species.

## Background

Elasmobranchs have been a subject of public curiosity since the first specimens were displayed in public aquaria and marine biological stations in the 1860s. These structures, along with research efforts and the development of display feeding methods, have provided opportunities to observe elasmobranchs and document their behavioural patterns in captivity (Koob, 2004). The discoveries have served as the foundation for further understanding their anatomy, metabolism, feeding habits and environmental requirements. This chapter will describe those points, focusing on the families of benthic and pelagic sharks (including the nurse shark, the tawny nurse shark, the zebra shark and the grey reef shark) having similar characteristics (Fig. 1).

To better understand the feeding requirements of the elasmobranch species kept in an aquarium, it is necessary to know the nutritional values of the main components. Examples include the protein to energy ratio, the proportion of carbohydrates, lipids, vitamins and minerals in their food. The optimal diet for a captive shark is a copy of its diet in the wild, both in quantity and quality (Janse et al., 2004a, 2004b). Current dietary vitamin and trace mineral recommendations for sharks are mainly based on those determined in the wild. Feeding in captivity is mostly done with pre-frozen food, however, it has its benefits as well as disadvantages. Firstly, it is crucial to eliminate possible parasites from the food and keep feed structure to achieve a

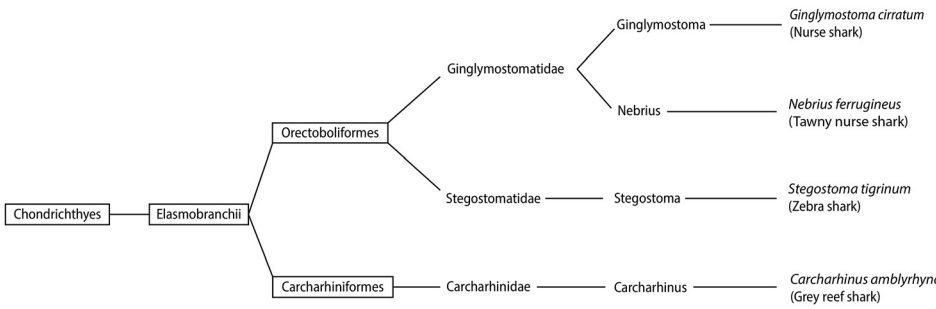



*Fig. 1.* Simplified taxonomic tree of the four species of sharks present in the Budapest Zoo and Botanical Garden (information extracted from ITIS/Integrated Taxonomic Information System: Bonnaterre, 1788; Lesson, 1831; Hermann, 1783; Bleeker, 1856; Moorhead et al., 2020)

continuous availability of food. Moreover, the transport and storage of feed can result in quality reduction, making the use of supplements, such as minerals and vitamins necessary, even life-dependent for the animals (Hoopes, 2017).

## Proteins

Pelagic sharks require a diet in captivity that closely mimics their wild feeding habits, encompassing key nutritional components such as proteins, carbohydrates, lipids, vitamins and minerals (Janse et al., 2004a, 2004b). Feeding pre-frozen food eliminates potential parasites and maintains the structural integrity of the feed, the transport and storage processes can lead to lower food quality, necessitating the use of supplements such as minerals and vitamins crucial for the well-being of the animals (Janse et al., 2004a, 2004b). Proteins play an essential role in fish nutrition as well, promoting growth and constituting a significant portion of body tissue. Fish efficiently utilize proteins, as they are ammoniotelic and excrete ammonia through gills, requiring less energy than ureotelic mammals (Paul, 2017). Bowen (1987) provides insights into the dietary protein requirements of fish, highlighting the importance of essential amino acids. A study by Jhaveri et al. (2015a, 2015b) on bonnethead sharks (*Sphyrna tiburo,* Linnaeus, 1758) indicates notably high aminopeptidase activity, a protein digestive enzyme, suggesting the significance of proteins in shark nutrition. The study also compares trypsin activity in different shark species, emphasizing the shared importance of proteins in their diet. The high-level activity of protein digestive enzymes across species implies their critical role in the nutrition of these animals, despite variations in metabolic rates (Jhaveri et al., 2015a, 2015b; Newton et al., 2015; Leigh et al., 2018).

On one hand, a study by Kim and Lee indicated that captive nurse sharks exhibited optimal growth and health when fed a diet comprising 55% protein mainly from fish and squid. The proteins in their diet came from both muscle tissue and exoskeletons of prey species. The amino acid composition is crucial, with a high emphasis on essential amino acids for tissue repair and growth such as lysine and methionine (Kim and Lee, 2018a, 2018b). On the other hand, investigations by Bernal et al. on blue sharks (*Prionace glauca*, Linnaeus, 1758), a pelagic species, demonstrated that diets containing 65% protein led to improved swimming performance and higher metabolic rates (Bernal et al., 2012a, 2012b). Finally, another study on great white sharks (*Carcharodon carcharias*, Linnaeus, 1758) revealed that a protein-rich diet (around 68%) was essential for their rapid growth rates and reproductive success, especially considering the long gestation periods and infrequent breeding cycles (Domeier and Nasby-Lucas, 2008).

## Lipids

In elasmobranchs, lipids serve a dual role, on the one hand as high-energy storage molecules and also as an integral component of cellular membranes. Fish exhibit high digestibility of lipids, with a preference over carbohydrates as an energy source (Newton et al., 2015). Fatty acids are essential components of the cellular membrane and are vital, as fish lack the capacity to synthesize them. Arachidonic acid, metabolized into prostaglandins, plays a role in follicle maturation and steroid production in female elasmobranchs during reproduction (Izquierdo et al., 2001). Omega-3 polyunsaturated fatty acids (PUFA) play a crucial role in vertebrate reproduction, influencing immune responses, inflammatory processes and the development of the brain and the eyes (Tocher, 2010). Arachidonic acid (AA) and omega-3 polyunsaturated fatty acids (PUFA) play significant roles in the reproductive physiology of sharks and other elasmobranchs. In female elasmobranchs, arachidonic acid is metabolized into prostaglandins, which are crucial for follicle maturation and steroid production. These prostaglandins, particularly PGE2 and PGF2α, help regulate the reproductive cycle, including processes such as ovulation and maintenance of the corpus luteum. For sharks, the intake of these fatty acids is crucial as it can impact prostaglandin and steroid levels directly influencing their reproductive capabilities. This underscores the importance of essential fatty acids in the habitats of these marine animals (Norambuena et al., 2013).

Blood sampled from the caudal vein of blacktip reef sharks (*Carcharhinus melanopterus,* Quoy and Gaimard, 1824) and nurse sharks revealed higher B-hydroxybutyrate levels during mating, while blacktip sharks exhibited increased corticosteroid concentrations. This suggests that nurse sharks may utilize B-hydroxybutyrate for energetic substrates during copulatory activities through lipid storage mobilization (Moorhead et al., 2020). Comparatively, European eels maintain a balance of PUFA n3 and n6 during spermiation, indicating mobilization of these molecules from the liver (Baeza et al., 2015).

Epipelagic sharks exhibit high metabolic rates due to their active and migratory lifestyles. They rely heavily on lipids (e.g. triacylglycerols and squalene) stored in their livers. These lipids provide a dense energy source necessary for long-distance swimming and capturing fast-moving prey (Pethybridge et al., 2014; Davidson et al., 2014). The ability to store and efficiently mobilize these energy reserves is crucial for their survival in the open ocean, where food availability can be unpredictable. In contrast, benthic sharks, like the nurse shark, have lower metabolic rates and a more sedentary lifestyle. They store lipids not only in the liver but also in muscle tissues. These sharks consume slower-moving or sessile prey influencing their lipid composition. The energy demands of benthic sharks are lower and their lipid stores are adapted to provide sustained energy over longer periods rather than rapid bursts. The cellular membranes of epipelagic sharks contain high levels of omega-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These PUFAs are crucial for maintaining membrane fluidity, which is vital for proper cellular function, particularly among the variable temperatures and pressures of the open ocean. This flexibility helps these sharks adapt to different environmental conditions encountered during their extensive migrations.

Benthic sharks, however, have a different fatty acid profile that includes a higher proportion of saturated fatty acids. These provide greater membrane stability, beneficial in the more constant and higher pressure ocean floor environments. The stable habitat of benthic sharks reduces the need for high levels of membrane fluidity, allowing for a lipid composition that supports their specific environmental needs (Davidson et al., 2014).

Studies also reveal that placental sharks are born with low concentrations of arachidonic acid (AA) and docosahexaenoic acid (DHA). This may be attributed to either low PUFA-provision during pregnancy or increased PUFA-utilization by pups during intrauterine development, highlighting the significance of lipid metabolism during this period (Lund et al., 2008).

During the mating season, adult nurse sharks exhibit elevated levels of B-hydroxybutyrate, a ketone body that serves as an important energy substrate. This rise suggests that nurse sharks mobilize their lipid stores to meet the increased energy demands of copulatory activities. B-hydroxybutyrate, produced through the breakdown of fatty acids, provides a sustained energy source that is crucial during periods of heightened activity when glucose reserves might be insufficient. Mobilizing lipids into ketone bodies like B-hydroxybutyrate enables nurse sharks to sustain prolonged mating efforts without immediate need for dietary replenishment. These metabolic adaptations illustrate the flexibility required for sharks to thrive in diverse ecological niches. The nurse sharks' lipid-based energy strategy is suited to their benthic environment, where energy reserves must be mobilized efficiently during sporadic feeding opportunities. Meanwhile, blacktip reef sharks benefit from a stress-induced glucose mobilization mechanism that supports their dynamic and transient reproductive activities in the epipelagic zone (Norambuena et al., 2013; Sorbera et al., 2001).

A study by Goericke et al. (2015) found that captive nurse sharks thrived on diets containing approximately 12% lipids, primarily sourced from fish and squid (Goericke et al., 2015). Investigations by Cortes and Gruber (2019) on Wobbegong sharks revealed that a diet with 10–15% lipids, derived from crustaceans and fish, was essential for their energy balance and overall health (Cortes and Gruber, 2019). Studies by Dickson et al. (2016) on blue sharks demonstrated that diets with 25% lipids led to optimal growth and energy efficiency, highlighting the importance of high-fat prey in their diet (Dickson et al., 2016). Research on great white sharks by Weng and Block (2004) revealed that a diet with around 28% lipids, derived from fatty fish and marine mammals, was critical for maintaining their energy levels and supporting their long-distance migrations. Benthic sharks, with their slower metabolisms and more sedentary lifestyles, require lower lipid proportions in their diets while pelagic sharks, due to their high activity levels and migratory behaviour, need higher lipid intake to support their energy demands and metabolic functions (Weng and Block, 2004).

## Carbohydrates

Carbohydrates constitute a fundamental component of plants, encompassing sugars, starches, gums, and cellulose. In animals, notably sharks, carbohydrates manifest as glycogen, sugars and their derivatives (Janse et al., 2004a, 2004b). Despite the identification of a relatively low level of maltase, an enzyme responsible for carbohydrate degradation, in the intestines of certain sharks, such as the bonnethead shark, these marine creatures often include crustaceans in their diet. Notably, crustaceans feature a substantial amount of chitin in their shells. The distinct dietary preferences of sharks, as exemplified by the bonnethead, are evident in their enzyme profiles. For instance, a twofold increase in the activity of B-glucosidase, observed in herbivorous teleost fish, suggests the capacity to digest cellulose and laminarin. Moreover, elasmobranchs exhibit elevated concentrations of N-acetyl-B-d-glucosidase (NAG), surpassing levels found in carnivorous teleost fishes by up to five times, particularly in the distal portion of their intestines. In sharks, NAG primarily functions in the digestive system, specifically within the intestinal tract. This enzyme aids in the breakdown of chitin, a major component of the exoskeletons of crustaceans and other marine organisms that constitute a substantial part of the diet of benthic sharks. By hydrolysing chitin into N-acetylglucosamine (GlcNAc) monomers, NAG facilitates the absorption and utilization of these breakdown products by the shark's digestive system (Smith and Jones, 2018). The distal intestinal tract of benthic sharks is specialized for the efficient absorption of N-acetylglucosamine (GlcNAc), the product of NAG activity. The absorption of GlcNAc is mediated by specific transport mechanisms that facilitate its uptake into enterocytes, the intestinal lining cells. Once internalized by enterocytes, GlcNAc can be channelled into various metabolic pathways:

1. Energy production: GlcNAc is converted into glucose-6-phosphate, which subsequently enters the glycolytic pathway, providing energy for the shark (Lee and Kim, 2017).
2. Glycoprotein and glycolipid synthesis: GlcNAc acts as a precursor in the synthesis of glycoproteins and glycolipids, which are essential for cellular communication, immune response and the maintenance of tissue integrity (Martinez et al., 2021).

Understanding the role of N-acetylglucosaminidase (NAG) in the digestive processes of benthic sharks holds significant implications for veterinary medicine. Providing a diet that optimizes NAG activity is crucial for the health and longevity of sharks in captivity or under veterinary care (Jackson and Lee, 2020). Moreover, monitoring NAG activity can be utilized as a diagnostic tool to evaluate digestive efficiency and overall health status in these marine animals (Gonzalez and Peters, 2019).

The activity levels of each enzyme type are likely to vary depending on the specific dietary habits of the particular shark species. Consequently, delving deeper into the types of enzymes, their roles and modes of action could significantly

contribute to understanding the metabolism and nutritional requirements of individual shark species. This deeper comprehension facilitates the advancement of nutritional strategies aimed at mimicking the diet in their natural habitats (Janse et al., 2004a, 2004b).

A study by Kim and Lee (2018a, 2018b) found that nurse sharks in captivity performed well on diets containing about 7% carbohydrates, sourced from squid and fish muscle glycogen (Kim and Lee, 2018a, 2018b). Stevens et al. (2016) reported that Wobbegong sharks showed optimal energy levels and metabolic rates when their diet included 5–8% carbohydrates, derived mainly from their crustacean prey (Stevens et al., 2016). Bernal et al. (2012a, 2012b) found that blue sharks maintained high metabolic performance with a diet containing 6–8% carbohydrates, derived from the muscle glycogen of fish and cephalopods (Bernal et al., 2012a, 2012b). Domeier and Nasby-Lucas (2008) observed that great white sharks required around 7% carbohydrates in their diet for optimal energy and metabolic functions, sourced from their high-fat, high-protein prey. In captivity, it is crucial to ensure that the carbohydrate content in the diet of sharks does not exceed their natural dietary intake to prevent metabolic disorders. The natural diets of both benthic and pelagic sharks provide sufficient glycogen, ensuring their carbohydrate needs (Domeier and Nasby-Lucas, 2008).

## Minerals

It is widely presumed that the dietary requirements for minerals in elasmobranchs are very similar to the needs of teleost species. Nevertheless, they are very difficult to establish because those minerals are required only in trace amounts and that some are absorbed via the environment (aqua) through the gills. It is also important to note that significant interactions between mineral-mineral and mineral-vitamin also exist (Janse et al., 2004a, 2004b) and complexes can be formed, interfering with the actual diet of the animal. For example, the amount of copper (Cu) required depends on the concentrations of water-borne minerals, such as zinc (Zn), iron (Fe), and molybdenum (Mo), which can interfere with Cu-absorption (NRC, 2011).

The essential minerals in fish nutrition (Wedemeyer, 1996; NRC, 2011) are summarized in Table 1.

Sharks, both benthic and pelagic, require a range of minerals for critical physiological functions such as growth, reproduction and maintaining homeostasis. Essential minerals include calcium and phosphorus, which are crucial for the formation and maintenance of their cartilaginous skeletons. This requirement is especially significant for benthic sharks like the nurse shark, which often have more robust skeletal structures to support their sedentary lifestyle on the ocean floor. In contrast, pelagic sharks like the great white need a lighter skeleton to enhance buoyancy and agility in open waters (Smith, 2019). Magnesium is another essential mineral, playing a vital role in enzymatic reactions, muscle function, and the synthesis of proteins and nucleic acids. This is particularly important for pelagic sharks that engage in long-distance migrations and require efficient muscle function for sustained swimming. Benthic sharks, although less migratory, still require magnesium for localized movements and metabolic processes (Jones et al., 2020). Sodium and potassium are critical electrolytes for maintaining osmotic balance and proper nerve function. Benthic sharks, which often inhabit environments with varying salinity levels, such as estuaries and coastal regions, must effectively regulate these electrolytes to adapt to their changing surroundings. Pelagic sharks, swimming in the relatively stable open ocean, also need these minerals but face different challenges related to long-distance travel and deeper dives (Doe and Roe, 2021). Overall, while the fundamental mineral requirements are similar, the specific needs and

*Table 1.* Minerals, deficiency signs and requirements per kg of dry diet for fishes (adapted from Chow and Schell, 1980; NRC National Research Council, 2011)

| Mineral | Min and maximum requirement in kg/dry diet | Average requirement in kg/dry diet | Deficiency signs |
|---|---|---|---|
| Calcium | 2–15 g | 8.5 g | Impaired growth, hard tissue mineralization |
| Phosphorus | 5–10 g | 7.5 g | Impaired growth, reduced hard tissue mineralization, lipid gain |
| Chloride | 1–5 g | 3 g | Impaired growth |
| Potassium | 2–3 g | 2.5 g | Convulsions |
| Sodium | 1–3 g | 2 g | Impaired growth |
| Magnesium | 0.5–0.7 g | 0.6 g | Muscle convulsions, weakness |
| Iron | 30–200 mg | 115 mg | Impaired growth, anaemia |
| Zinc | 20–150 mg | 85 mg | Impaired growth, cataracts, skeletal deficiencies |
| Manganese | 7–13 mg | 10 mg | Impaired growth, skeletal deformities |
| Cobalt | 5–10 mg | 7.5 mg | Anaemia |
| Copper | 1.5–5 mg | 3.25 mg | Impaired growth |
| Iodine | 0.6–1.1 mg | 0.85 mg | Goitre |
| Selenium | 0.1–0.7 mg | 0.4 mg | Impaired growth, anaemia |

adaptations differ between benthic and pelagic sharks due to their distinct lifestyles and habitats. Understanding these differences is critical for their conservation and management, especially in the context of captive species.

The predominant mineral deficiency observed in elasmobranchs, particularly in sharks, is iodine deficiency, a condition that, under specific conditions and levels, can result in goitre (Janse et al., 2004a, 2004b). This underlines the general inadequacy of minerals absorbed from water to fulfil the overall dietary requirements. Consequently, supplementation through the diet becomes imperative, whether achieved through natural food sources or feeds containing these elements. Meeting this mineral requirement is pivotal for ensuring normal growth and survival and is also essential to physiological cellular metabolism (Paul, 2017).

## Vitamins

Approximately 15 vitamins are identified in fish, with symptoms of deficiency in elasmobranchs often inconsistently manifested, complicating identification until necropsy or histopathology (NRC, 2011). Determining precise vitamin requirements for captive sharks is challenging, given the diverse parameters (sex, age, species, environment) which influence their needs (Leigh et al., 2017). While the NRC (2011) offers a comprehensive list of vitamin amounts for sharks, the exact vitamin requirements in captivity remain elusive (Table 2).

Vitamin A is crucial for vision, growth and immune function. Benthic sharks, such as the nurse shark, often inhabit murky waters where vision is paramount for hunting and navigation. Therefore, adequate vitamin A intake is vital for maintaining their retinal health and overall visual acuity. Pelagic sharks like the great white, also rely on vitamin A but face different challenges, such as long-distance vision in clearer waters (Johnson, 2018). Vitamin D plays a pivotal role in calcium absorption and bone health. For benthic sharks, which may have less exposure to sunlight due to their bottom-dwelling habits, dietary sources of vitamin D are especially important. This ensures they can maintain their cartilaginous structure and overall skeletal integrity. Pelagic sharks, exposed to more sunlight during their long migrations, might synthesize more vitamin D naturally but still need dietary sources to meet their requirements (Williams and Smith, 2020). Vitamin E acts as an antioxidant, protecting cellular membranes from oxidative damage. This is particularly crucial for pelagic sharks that undergo strenuous physical activity during long migrations. The high metabolic rate associated with continuous swimming increases the production of free radicals, thus necessitating a robust antioxidant defence system. Benthic sharks, with a generally more sedentary lifestyle, still require vitamin E but in different proportions relative to their activity levels (Harris et al., 2019). Vitamin C is essential for collagen synthesis, wound healing and immune function. Given the benthic sharks' frequent interactions with the ocean floor, which can lead to abrasions and injuries, an adequate supply of vitamin C is critical for efficient wound healing. Pelagic sharks also require vitamin C, especially to maintain their skin function and overall health during extensive travels through varying environmental conditions (Clark and Martin, 2017a, 2017b, 2017c).

Aquarium shark diets primarily consist of frozen food for transport, parasitological concerns, and quality maintenance, and nutrient loss (De Silva and Anderson, 1995). Supplementation becomes necessary, typically preceding

*Table 2.* Vitamins, signs of deficiency and requirements per kg of dry diet for fishes (adapted from NRC National Research Council, 2011)

| Vitamin | Min and maximum requirement in unit/kg dry diet | Average requirement in unites/ kg dry diet | Deficiency signs |
|---|---|---|---|
| A | 6,600–13,000 IU | 9,800 IU | Ascites, anorexia, spinal deformities, oedema, erosion, eye pathology, lethargy |
| Choline | 400–3,000 mg | 1,700 mg | Fatty liver |
| D | 400–2,400 IU | 1,400 IU | Fatty liver, spasms |
| Myoinositol | 166–500 mg | 333 mg | Erosions, fatty liver, lethargy |
| C | 100–500 mg | 300 mg | Abnormal swimming, ascites, anorexia, erosion, spinal deformities, eye pathology, lethargy, loss of equilibrium |
| Niacin (B3) | 12–150 mg | 81 mg | Abnormal swimming, lethargy, spinal deformities, oedema, muscle weakness |
| Riboflavin (B2) | 3–20 mg | 11.5 mg | Abnormal swimming, spinal deformities, erosion, eye pathology, lethargy |
| Pyridoxine (B6) | 0–20 mg | 10 mg | Lethargy, spasms |
| Thamin (B1) | 1–10 mg | 5.5 mg | Loss of equilibrium, spasms |
| Folic acid | 1–10 mg | 5.5 mg | Haematological changes, lethargy |
| Biotin | 0.05–2.5 mg | 3.75 mg | Abnormal swimming, fatty liver, lethargy, spams |
| K | 0.5–2 mg | 1.25 mg | Haemorrhages, prolonged blood clotting |
| Cyanocobalamin (B12) | 0.01–0.05 mg | 0.03 mg | Anaemia, haematological changes, anorexia |

feeding or incorporation into pre-frozen feed. While most teleost species lack vitamin C synthesis capability, elasmobranch studies suggest some species do possess the necessary enzyme (Mæland and Waagbø, 1998).

Dosing methods of vitamins and minerals greatly vary between different institutions (Table 3). A survey was completed by 70 institutions and solicited information on the types and percentages of dietary items fed to aquatic animals as well as the type and dosing method of vitamin and mineral supplements provided (Mazzaro et al., 2016).

Commonly, vitamins and minerals are added through the food, but some aquaria also use the water in the tank as a supplement base: powder, liquid or tablets can be utilized in this fashion. Using powder spread on the food can alter its taste and vitamin integrity can be easily lost. Another technique which can be implemented is the injection of a liquid mixture into the food itself. The most common and successful way is still the usage of tablets via the oral or abdominal cavity, or under the skin of the feed item. Finally, the vitamins can be injected intramuscularly (IM.) during medical procedures (Janse et al., 2004a, 2004b). Regarding the type of supplementation chosen, instructions can be given for dosing according to the body mass (BM) or based on the amount of feed offered. The first technique requires the frequent weighting of the animal, which can be stressful and requires special preparation (Janse et al., 2004a, 2004b; Mazzaro et al., 2016).

The second technique, the administration of tablets through the oral or abdominal cavity, or subcutaneously in the feed item, is not precise enough and largely depends on dietary changes, meaning that adjustment will be needed to maintain a proper range of well-known supplementation (Janse et al., 2004a, 2004b). Several companies have developed vitamin/mineral supplement mixtures, varying in quality and the quantity required per animal. In a survey, 13 aquaria (out of 15), added vitamins produced by companies to their shark's diet. Six of them created their own vitamin mix and most of the aquaria (10) added iodine to their food, while only two added it to the water (Janse, 2003). The composition of vitamin mixes (in $\mu g^{*}kg^{-1}$ food$^{*}$week$^{-1}$ or $mg^{*}kg^{-1}$ food$^{*}$week$^{-1}$) used for the dietary supplementation of demersal sharks in 10 European public aquaria can be found in Table 3. Table 3 shows the method of supplementation to elasmobranchs based on survey results. A study shows the large diversity of techniques and the type of supplementation used, proving again that very little research has been done on what dosages are the best in terms of success rate and it is the choice of the given institution to decide which method suits best for their captive species. The objective is to augment the health and longevity of these fish by replenishing any lost vitamins and minerals. The approach taken involves dosing the supplements based on the weight of the food rather than the bodyweight of the elasmobranchs. Two tablet sizes are offered: one tablet per 250 g of food (large) and one tablet per 25 g of food (small). Administration of these tablets is most conveniently performed by inserting them into the fish's oral cavity, beneath the operculum (gill cavity), within the fish's abdominal region, or by creating a minor incision in the skin or muscle and inserting the tablet. Notably, these tablets boast a shelf life of three years and can be stored at ambient temperature (Jones, 2023).

*Table 3.* Method of supplementation to elasmobranchs based on survey results. The survey was completed by 70 institutions and solicited information on the types and percentages of dietary items fed to aquatic animals as well as the type and dosing method of vitamin and mineral supplements provided (Mazzaro et al., 2016)

| Supplement dosing regime | Elasmobranchs |
|---|---|
| By diet | 54% |
| By body weight | 0% |
| Specified number of tablets/days | 21% |
| Thiamine and vitamin E only | 0% |
| Additional supplements | 14% |
| No supplements | 7% |

## Transitioning from the natural habitat to a captive environment: exploring the origins, elements and repercussions of primary nutritional ailments in sharks held in aquariums

Nutritional diseases are commonly found in captive sharks, because those animals need to adapt from a natural wild environment to a captive world in aquarium, requiring their metabolism, physiology and nutritional habits to accommodate accordingly. Defective nutrition is considered as one of the most important elements in the development of non-infectious (deficiency, excess, imbalance) and infectious (parasitological, bacteriological, fungal or viral) diseases for captive wild species. Today, the expansion of aquaculture and more precisely, of aquaria, requires continuous improvement in nutritional formulation and fish technology (OIE glossary, 2018). Nutritional disorders can arise for diverse reasons: nutritional imbalance (including vitamin and mineral deficiencies), incorrectly stored food, vitamin deficiency, infected live food and feed toxicity (for example mycotoxins). However, biological stress is another parameter that seems to be essential in the emergence of this unfavourable imbalance, especially during a change of environment from wild to captive. Stressors can be biotic (relationship with spatial environment, transport, handling, water quality) and abiotic (conflict, diseases due to nutrition or pathogens). Determining those stressors and their origin will have a considerable impact on the treatment of most diseases. Stressors encompass a range of factors such as hyperactivity, physical harm, alteration of the external surroundings, engagements in diving activities and encounters with humans during the processes of capture, transportation and other procedures related to the care and management of the organism (Smith, 1992). This is why minimizing potential stressors in the environment of captive sharks is a key point to reduce the risk of any diseases and increase their survival (Schlaff et al., 2014).

## Vitamin A hypo- and hypervitaminosis

As a fat-soluble vitamin, vitamin A can accumulate in the body. When vitamin A is ingested orally, it will take the form of retinyl esters and be transformed into retinol in the enterocytes. It will then be stored in adipose tissue and organs such as kidney and the adrenal glands and will be eliminated by the kidney or into the bile via the liver. It seems hence inevitable to understand that vitamin A takes a central place in the physiological processes of sharks and directly affects vision, reproduction, embryogenesis, growth and differentiation/maintenance of epithelial cells (Dierenfeld et al., 1991).

Thanks to studies conducted in the 20th century, there is a consensus that the metabolic functions of vitamin A in fish are the same as those of other vertebrates (Halver, 2002).

Vitamin A hypo/hypervitaminosis is well described in the literature in fish but not clearly documented in elasmobranchs, more precisely sharks and this is why the following review will be expanded to fish in general to give a more open idea of the actual concept (Thompson et al., 1994; Hernandez and Hardy, 2020).

## Causes of vitamin A deficiency/excess

Vitamin A losses occur mainly due to technical errors occurring during food transport, storage, preparation, or due to nutritional problems such as an imbalance in the dietary requirements. Finally, but less likely, vitamin A can become deficient if the animal encounters metabolic problems (malabsorption, deficit in storage, maldigestion). On one hand, it is important to remember that in captivity, animals may not feed on a balanced diet. This is why adding vitamins and other trace elements seems essential to achieve the most adapted diet possible. On the other hand, excess of vitamin A can be a consequence of an over-supplementation of the added vitamins. It is also important to highlight that in general, the concentrations of vitamin A in whole fish are already typically quite high (Dierenfeld, 1991).

## The physiological mechanism of vitamin A

Thanks to studies conducted in the 20th century, there is a consensus that the metabolic functions of vitamin A in fish are the same as those of other vertebrates (Halver, 2002). The mechanism is presented below (Fig. 2):

## Consequences of vitamin A imbalance

***Vision.*** Vitamin A plays a crucial role in shark vision by facilitating the production of 11-cis-retinal, a photosensitive chromophore essential for the visual pigments in their retinal rods and cones (Hernandez and Hardy, 2020). Vitamin A deficiency in sharks can result in conditions such as night blindness and reduced visual acuity due to insufficient 11-cis-retinal production. Conversely, excessive vitamin A can lead to structural damage to the retina and optic nerves, causing symptoms like blurred vision, photophobia, exophthalmia and depigmentation (Aoe et al., 1969).

***Systemic function.*** All-trans-RA and 9-cis-RA regulate gene expression which are receptor mediated. Retinoids can directly initiate the synthesis of a protein after binding on nuclear receptors or indirectly by producing transcription factors that stimulate target genes, thus enhancing growth hormone, oxytocin, cell growth factors, enzymes and protein of extracellular matrix (Hernandez and Hardy, 2020). Imbalances in vitamin A levels in sharks can lead to systemic issues such as ascites, anorexia, decreased growth and lethargy (Janse et al., 2004a, 2004b).

***Reproduction.*** RA gradients have recently been linked to the regulation of the expression of proteins involved in meiosis (Ruivo et al., 2018). Marine fish eggs contain 70% Ral and the remaining consist of Rol (Retinol) (Lubenz et al., 2003).

RA gradients are crucial for regulating proteins involved in meiosis in sharks. Marine fish eggs, including those of

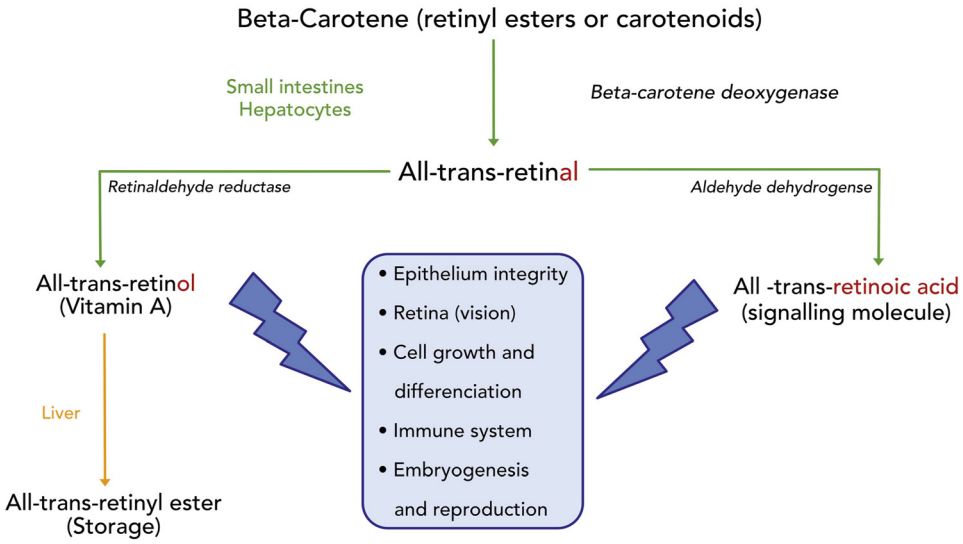



*Fig. 2.* Simplified pathway of the vitamin A metabolism and functions (Godoy Parejo et al., 2019; Dewett et al., 2021)

sharks, contain significant amounts of Retinal (Ral) and Retinol (Rol) (Lubenz et al., 2003). Both hypo- and hypervitaminosis A can impair reproductive performance and increase the embryonic death rate in sharks. Excessive vitamin A disrupts normal embryonic development, leading to teratogenic effects such as deformities, spinal anomalies, craniofacial malformations in shark larvae (Janse et al., 2004a, 2004b). Excess RA levels, resulting from hypervitaminosis A, can disrupt normal retinoid signalling pathways, leading to dysregulated gene expression and improper tissue differentiation during embryogenesis. Finally, high levels of vitamin A can induce oxidative stress in developing embryos, leading to cellular damage and impaired viability. Oxidative stress can disrupt normal cellular processes, including DNA replication and repair, ultimately leading to embryonic death or developmental abnormalities (Blomhoff et al., 2006).

***Immune response.*** An elevated intake of exogenous vitamin A demonstrated a significant enhancement in phagocytic respiratory burst, bactericidal activity, lymphocyte functions, serum lysozyme and complement activities in Atlantic salmon (*Salmo salar*, Linnaeus, 1758) compared to those fed with low levels of vitamin A (Hernandez and Hardy, 2020). Young grass carp (*Ctenopharyngodon idella*, Valenciennes, 1844) were subjected to different levels of dietary vitamin A, followed by an *Aeromonas hydrophila* injection challenge. The findings confirmed that hypovitaminosis A resulted in a notable reduction of fish growth performance, an increased occurrence of enteritis, a $^{\text{decline}}$ in innate humoral immune response within the intestine and exacerbation of intestinal inflammation. Conversely, vitamin A deficiency in sharks can compromise growth performance, increase susceptibility to infections and lead to a decline in the innate immune response, making them more vulnerable to diseases and environmental stressors (Zhang et al., 2016a, 2016b; Hernandez and Hardy, 2020).

## Requirements, prevention and treatment of vitamin A imbalance

To be able to identify any deficiencies, measuring blood nutrient parameters and comparing them to the reference ranges is necessary. A summary of the mean blood nutrient values for aquarium and wild elasmobranchs is given in the *Elasmobranch husbandry manual II*, however, only the zebra shark (in captivity), showing a mean value of 110.3 ng $mL^{-1}$ for vitamin A. Vitamin A requirements for fish species of commercial aquaculture can be used as a baseline and it appears that marine species have higher requirements than freshwater fish. Determining the exact vitamin A requirement for sharks in International Units (IU) can be challenging due to limited research on sharks. However, based on general guidelines for other vertebrates and considering their physiological similarities, a broad estimate can be provided. For example, for some fish species, the recommended vitamin A intake ranges from approximately 2,000 to 5,000 IU per kilogram of diet ($IU^{*}kg^{-1}$) to meet their nutritional needs for growth, reproduction and overall health. Considering this range and the metabolic similarities between fish and sharks, it is plausible to estimate a similar vitamin A requirement for sharks. However, given the unique biology and ecological niche of sharks, specific studies on their vitamin A metabolism and requirements would provide more accurate recommendations (Hoopes, 2017).

Many public aquaria supplement shark diets with a blend of vitamins and minerals, specifying the exact composition. Some aquariums utilize pre-formulated vitamin mixes brands, while others prefer to create their own custom blends. It is crucial to emphasize the considerable diversity observed in the quantity and quality of these dietary supplements. In terms of vitamin A supplementation, the established range spans from 0.8 to 70 $mg^{*}kg^{-1}$ $food^{*}week^{-1}$, considering that 1 µg of retinol is equivalent to 3,333 IU of vitamin A (Janse, 2003). The calculation of supplement ranges requires factoring in the vitamin content of various food items. Depending on factors such as diet composition, animal weight and feeding frequency, a formula can be devised to ascertain appropriate vitamin supplementation. Janse et al. (2004a, 2004b) mentioned a range of 2,000–2,500 $IU^{*}kg^{-1}$ of diet of vitamin A for elasmobranchs. It is noteworthy that current dietary recommendations for vitamins and trace minerals for elasmobranchs often draw from those established for teleost fish (De Silva and Anderson, 1995). Great white sharks in captivity have benefitted from diets with 7,000 $IU^{*}kg^{-1}$ of vitamin A, supporting their vision and health (Domeier and Nasby-Lucas, 2008). Blue sharks have shown improved health markers and avoided deficiencies with a diet containing 6,000 $IU^{*}kg^{-1}$ of vitamin A. Nurse sharks in captivity have shown optimal health and immune function when their diet included around 3,000 $IU^{*}kg^{-1}$ of vitamin A, and Wobbegong sharks maintained good health and avoided vitamin A deficiency symptoms with a diet containing 2,500 $IU^{*}kg^{-1}$ (Lall and Kaushik, 2021). Benthic sharks require lower amounts of vitamin A (2,000 to 5,000 $IU^{*}kg^{-1}$) compared to pelagic sharks (5,000 to 10,000 $IU^{*}kg^{-1}$) due to their different activity levels and dietary sources. Both groups need sufficient vitamin A to support their physiological functions, but the source and amount vary according to their natural habitat and lifestyle. Toxicities resulting from excessive exposure to fat-soluble metabolites, like vitamin A, are rare. Nevertheless, a substantial body of literature suggests the potential for toxic accumulation of this metabolite (NRC, 1987; Russell, 2000).

## Iodine deficiency or goitre

Iodine is a chemical element found in seawater around 0.04–0.06 $mg^{*}L^{-1}$ (Morris et al., 2012). It can help regulate the total oxygen use. Iodine appears as an essential component of the elasmobranchs' metabolism, particularly of their thyroid glands, since the thyroid gland will assimilate and concentrate the iodide ion originating from the feed and from the environment. The thyroid gland produces

hormones that regulate metabolism, growth and development. In elasmobranchs, just as in other vertebrates, iodine is a necessary component for the synthesis of these thyroid hormones. Maintaining appropriate iodine levels in their environment helps ensure proper thyroid function and overall metabolic health in elasmobranchs. In the thyroid gland, it will be converted into triiodothyronine (T3) and thyroxine (T4) from the amino-acid L-tyrosine taken from the bloodstream. To allow their synthesis, storage and release, thyroid hormones exert an influence on growth and maturation and possess regulatory functions in the metabolism of energy (Lloyd, 1994). Tissues that are sensitive to thyroid hormones possess exclusively T3 receptors. Consequently, only T3 exhibits biological activity, while T4 functions as a prohormone that can be enzymatically converted into T3 (Leary et al., 1999). If the ingested food has an insufficient level of iodine, the thyroid gland will have reduced T3 and T4 production resulting in a lack of tyrosine binding to those hormones. Consequently, a negative feedback in the up-regulation of the TSH by the pituitary gland as well as of the TRH on the hypothalamus will arise. The result will be an elevation of TSH in the bloodstream (Fig. 3). The exacerbation of a goitre, for instance, may result from a synergistic interplay of factors, with a common combination being an iodine deficiency compounded by the presence of a goitrogenic agent (Crow, 2004). If a depletion or insufficiency in iodine is observed, the thyroid gland can develop a swelling/trauma, commonly defined as goitre. When the tissue is left in a state of iodine depletion, it has the potential to progress into a tumour and ultimately transform into a malignant tumour that generates recurring metastasis in various organs (Straub, 1993). An enlargement (hypertrophy) or an increase in the number of cells (hyperplasia) can both lead to a goitre. Although this condition is frequent and well documented in sharks, unfortunately the actual causes and processes are poorly understood (Crow, 2004). During a study, thyroid hormone concentrations of the whitetip reef shark (*Carcharhinus longimanus*, Poey, 1861) were measured and compared between healthy and goitred individuals (Table 4). Results showed a decreased production of T3 and T4, indicating that individuals with goitre are hypothyroid. Several possible causes can explain this condition, including decreased iodine concentration of the water. Water management of the tank should include monitoring iodine levels since protein skimmers have a main effect on it. Crow et al. (1999) emphasized that the analysis of seawater in aquariums often indicates a diminished presence of iodide and an elevated level of nitrogen, specifically nitrate. Most of the aquaria with goitred sharks have less than 0.006 $mg^{*}L^{-1}$ on this element in the tank water. In the same facility, it was reported that iodine concentration, in combination of iodide and iodate, was the same as the natural seawater, which then indicates that iodide plays a crucial role in the enhancement of goitres (Crow et al., 1999). Hunt and Eales (1979) found that iodide uptake was at least 84% from surrounding water and 16% from diet in the rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792), however, the percentage of iodide uptake in elasmobranchs is unknown (Hunt and Eales, 1979; Crow et al., 1998).

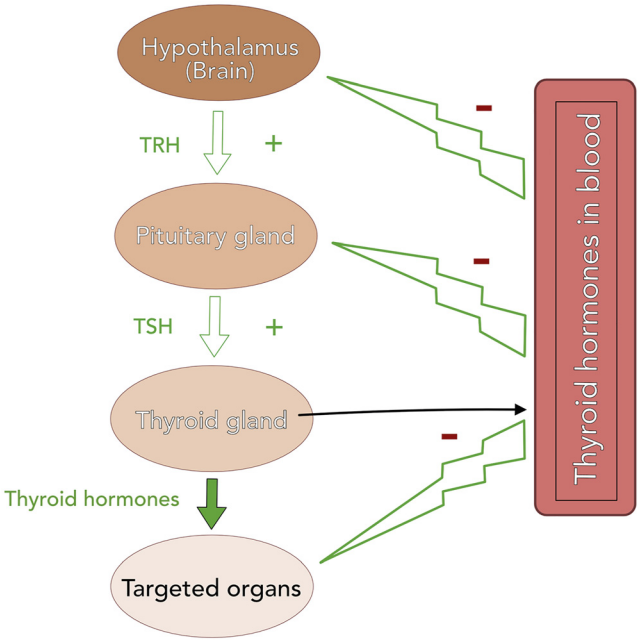



*Fig. 3.* Hypothalamic control over the thyroid gland. TRH: thyroid releasing hormone, TSH: thyroid stimulating hormone
*Note:* T4 (thyroxine) will act as negative feedback on the anterior pituitary gland, while T3 (triiodothyronine) will have a negative feedback role on the hypothalamus.

## Causes of iodine deficiency

Goitrogenic substances, such as ammonia, urea, nitrate etc. acting as environmental toxins may affect the thyroid gland, therefore contributing to iodine deficiency (Crow, 2004; Morris et al., 2012). Elevated concentrations of ammonia, a nitrogenous waste product excreted by sharks primarily through their gills, can result from compromised water quality or excessive organic waste accumulation. Such conditions are known to induce stress and physiological challenges in sharks. Notably, ammonia toxicity has been implicated in the disruption of thyroid hormone synthesis and secretion. This perturbation of thyroid function may precipitate thyroid dysfunction, subsequently contributing to metabolic dysregulation and broader health complications in sharks (Harbison, 1983). Sharks possess a unique adaptation known as urea-based osmoregulation, which allows them to maintain proper water balance in their bodies

*Table 4.* Comparison of the plasma blood values T3 and T4 between captive healthy sharks and goitreed sharks (Gerald L. Crow et al., 1999)

| | Healthy captive | Goitered captive |
|---|---|---|
| Serum T3 in $ng^{*}mL^{-1}$ | 0.52–0.83 | 0.22–0.33 |
| Serum T4 in $ng^{*}mL^{-1}$ | 1.34–8.77 | 0.93–0.99 |

despite living in a hyperosmotic environment. Urea, a breakdown product of protein metabolism, is actively retained in the shark's body to counteract the osmotic effects of seawater. However, excessive urea levels, either internally or in the environment, can disrupt thyroid function. Urea accumulation may interfere with iodine uptake or thyroid hormone synthesis, leading to thyroid dysfunction and associated metabolic disturbances (Mandelberg, 2020). Finally, nitrate can interfere with thyroid function indirectly through its conversion to nitrite. Nitrite can disrupt thyroid hormone synthesis by inhibiting the activity of thyroid peroxidase, an enzyme essential for iodine incorporation into thyroid hormones. Consequently, nitrate-induced thyroid dysfunction can contribute to metabolic disturbances and health issues in sharks (Lee and Jones, 1999).

A study conducted on 10 juvenile whitespotted bamboo sharks (*Chiloscyllium plagiosum*, Bennett, 1830) confirmed the role of nitrate in goitre development. During the study, half of the specimens were exposed to water with low nitrate levels and the other half to water containing high levels of nitrate, less than 1 $mg^{*}L^{-1}$ and more than 70 $mg^{*}L^{-1}$, respectively, for 29 days (Morris et al., 2012). The histological findings showed hyperplastic goitre developed in individuals living in high-nitrate environments, which is generally true for aquaria using recirculating water systems. Furthermore, if the water ozone is not controlled and kept at a tolerated level (0.02–0.03 $mg^{*}L^{-1}$), it may decrease the bioavailability of iodine, therefore, it is necessary that iodine levels are subjected to regular testing (Sherrill et al., 2000; Morris et al., 2012). Ozone level must be controlled regularly to keep it at the tolerable level. In a study conducted at the Blackpool Sea Life Centre, six shark specimens showed advanced states of goitre. Comparative results showed 0.01 $mg^{*}L^{-1}$ and 0.06 $mg^{*}L^{-1}$ iodine in the aquarium system and in the Irish Sea, respectively (Lloyd, 1994).

Benthic shark in aquaria such as *G. cirratum* or *Stegostoma tigrinum* are generally fed with whiteleg shrimp (*Litopenaeus vannamei*, Boone, 1931), squid (*Loligo* spp.) or Atlantic salmon (*S. salar*) which are a good source of iodine. Nevertheless, it is also important to note that some aquaria also use low iodine food items such as herring (*Clupea harengus*, Linnaeus, 1758) and smelt (*Smerus* spp.) for captive elasmobranchs which can lead to malnutrition. Most of the institutes keeping sharks use frozen food, which has the disadvantage of the loss of nutrients, particularly molecules such as iodine. This is why it seems unavoidable to supplement the food with iodine tablets using a mixed formula. Agrawal and Mahajan (1981) described ascorbic acid deficiency reducing iodine uptake.

## Consequences and histological assessment of goitre in elasmobranchs

To have a better understanding of the different histological changes resulting from a goitre, being able to recognize a healthy thyroid tissue is essential. Healthy thyroid gland presents as roundish follicles of various size, with a layer of single or stratified cuboidal (or columnar) epithelium without follicular hypertrophy or hyperplasia, accompanied with a profuse colloidal mass (Crow, 2004).

However, goitreed tissue histology can reveal 3 types of goitres in sharks. Diffuse colloid goitre: large round follicles filled with colloid together with smaller scattered follicles, from cuboidal to columnar. Papillary projections can be identified. Some of these untreated types of goitres can develop into multinodular colloid goitres (Crow, 2004). Multinodular colloid goitre: follicles varying in shape and size, from flattened to cuboidal to columnar. Nodularity made by hyperplastic follicles, where fibrous bands can be found (and can indicate a previous hemorrhage). Most enduring colloid goitres undergo a transformation into multinodular colloid goitres (Robbins, 1994; Crow, 2004). Diffuse hyperplastic goitre: mostly small to medium follicles with little or no colloid and columnar epithelium (Crow, 2004). If iodine deficiency endures without detection and remains untreated over time, the consequences of the goitre become discernible not only at the internal tissue level, as observed in histological samples, but also manifest prominently during external examinations through distinct clinical signs (Lloyd, 1994; Crow et al., 1998).

## Clinical signs and symptoms of goitre in elasmobranchs

In advanced cases of goitre, individuals can show swelling under the midline of the jaw, which can reach up to 10 times the size of a healthy thyroid gland (Fig. 4) (Morris et al., 2011). Clinical signs will result in general symptoms like inappetence due to an improper capability to eat which can eventually result in death. Finally, since thyroid hormones play an important role in the growth of juvenile animals, the hypothesis stands that a long-term presence of an untreated goitre in young individuals could affect their development. Further research is needed to confirm this theory (Morris et al., 2012).

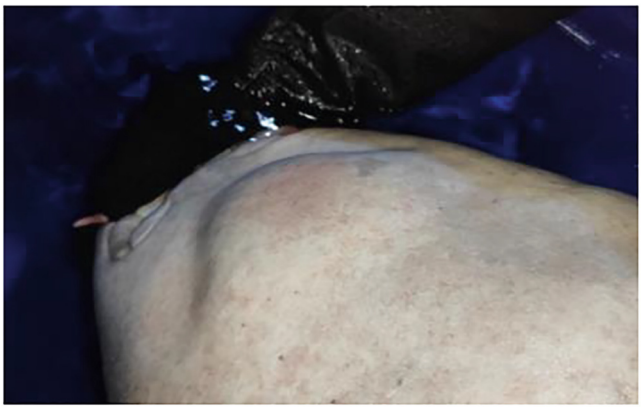


*Fig. 4.* Nurse shark (*Ginglymostoma cirratum*) with a goitre (Hoitsy, 2023)

## Requirements, prevention and treatment of iodine deficiency

The first step in avoiding the onset of goitre in captive sharks is knowing the recommended requirements and the basic data needed to prevent iodine deficiency. Table 5 groups the main values found in the literature to prevent the development of this pathology. This guideline needs to be adapted to the types of aquaria (water chemistry and species density), to animal characteristics (species, reproductive condition, diet) and health status. The prevention and treatment of goitre involve three primary strategies, namely, the implementation of water changes, a process that may extend over a prolonged period of time, supplementation of iodine solution into the tank water or integration of iodine solution into the diet to achieve optimal levels and the utilization of formulated vitamin and mineral preparations (Uchida and Abe, 1986).

Both benthic and pelagic juveniles need sufficient iodine to support the synthesis of thyroid hormones, which are critical for proper development and metabolic regulation. Iodine deficiency in juvenile sharks can lead to stunted growth and developmental abnormalities (Williams et al., 2020a, 2020b). In adult sharks, reproductive status significantly influences iodine needs. Pregnant and breeding females have increased iodine requirements to support the development of embryos and maintain their own metabolic health. This is true for both benthic and pelagic species. Adequate iodine intake during pregnancy is crucial for the successful development of offspring and overall reproductive success (Clark and Martin, 2017a, 2017b, 2017c).

Successful prevention of goitre in sharks can be enhanced by considering factors such as age, species and season. Research indicates that immature sharks exhibit lower levels of T4 and T3 compared to pregnant individuals, with no significant variance based on sex (Volkoff et al., 1999). Additionally, studies show that T4 concentrations in sharks are nearly halved during summer, compared to winter. Consequently, supplementing iodine, particularly in immature sharks and during the summer season, could serve as an initial preventive measure against goitre formation (Crow et al., 2001).

Subsequent investigations have revealed a regression of goitre in sharks inhabiting natural seawater lagoons (Crow et al., 2001). Within the Ueno Park Aquarium in Tokyo, artificial regulation of iodine levels is achieved through the introduction of potassium iodide (KI) into the water tank. Precision in maintaining the iodine level at 0.1 $mg^{*}L^{-1}$ ensures a consistent elevation beyond natural seawater concentrations, thereby preventing goitre development and inducing regression, as observed in a sandbar shark (*Carcharhinus plumbeus*, Nardo, 1827) (Uchida and Abe, 1986). In instances of goitre manifestation, administering oral iodine (KI) treatment at a dosage of 10 $mg^{*}kg^{-1}$ BW was efficient. Nevertheless, caution is necessary when introducing iodine into the aquatic environment, given that the solvation of KI may lead to perilous concentrations, potentially proving harmful to fish populations (Stoskopf, 1993).

As previously noted, iodine can be introduced into the diet through absorption in the gastrointestinal tract, typically in the forms of potassium iodide, sodium iodide or calcium iodate (Uchida and Abe, 1986; Miller and Ammerman, 1995). Certain institutions use iodine-rich algae as a supplement. The National Aquarium of Baltimore achieved successful goitre treatment by incorporating 6 mg of iodine per kg of fish body weight per week into the feed, establishing a foundational dosage (Lloyd, 1994). However, in facilities anticipating goitre occurrences, preventive administration of an iodine derivative is recommended. An ideal dosage falls within the range of 10–30 $mg^{*}kg^{-1}$ BW per week (Crow, 2004). Similarly, the Blackpool Sea Life Centre observed a 65% reduction in goitre swelling in nurse sharks by introducing iodine salt (40 mg KI*60 $kg^{-1}$ per week) into their diet. A comprehensive overview of the precise amounts and frequencies for each treatment compound is presented in Table 6, aiding the formulation of both prophylactic and therapeutic strategies (Lloyd, 1994).

## Hepatic steatosis or fatty liver syndrome

The hepatic organ, constituting 20% of a shark's body size, represents the largest gland, encapsulated with perfused homogenous sinusoids. In sharks, this organ consists of a left and a right lobe and between these two, a smaller caudate lobe (de Melo et al., 2019) (Fig. 5). The liver surrounds the stomach, intestines, spleen, and typically hosts the gallbladder attached to the right lobe, opening into the duodenum for bile secretion. As a characteristic feature of vertebrates, the liver exhibits a central vein linked to hepatocytes, organized in sinusoidal structures. This central vein extends to the portal vein, forming the portal triad in conjunction with the hepatic artery and bile duct (Klimley, 2013; Matthews and Parker., 1950).

Within the shark's physiology, the liver serves as a principal repository for fats, gradually accumulating to constitute 60% of its final composition. In certain regions, shark liver oil is esteemed as a high-quality supplement,

*Table 5.* Recommended iodine, iodide, nitrate and ozone values for captive sharks ([2]Jones, 2023; [3]NSW government, no dates; [1]Crow, 2004; [4]Morris et al., 2011; [5]Morris et al., 2012)

| Compound | Iodine | Iodide | Nitrate | Ozone |
|---|---|---|---|---|
| Dosage (close to natural seawater levels) | [1]Around 60 $\mu g^{*}L^{-1}$ | [2]Around 0.06 $mg^{*}L^{-1}$ [3]minimum 0.02–0.0 $mg^{*}L^{-1}$ | [2]<10 $mg^{*}L^{-1}$ [4]maximum 70 $mg^{*}L^{-1}$ | [5]residual concentration between 0.01 ppm – 0.1 ppm |

*Table 6.* Treatments for goitre in elasmobranchs, showing compound, dosages and reporting institution. Both *Mazuri* Vita-ZU shark/ray and *Sea Tabs* refer to commercial supplements (Adapted from Janse, 2003)

| Compound | Dosage | Institution name |
|---|---|---|
| Calcium iodate | 1,087 mg of food/week | Mazuri Vita-ZU shark/ray |
| Calcium iodate | 0.03–0.05 mg*kg$^{-1}$ BW* week$^{-1}$ | Burger's Zoo |
| CLM01 | 1.5 mL* week$^{-1}$ for each specimen | Basel Zoo |
| Potassium iodide | 0.2 mg* L$^{-1}$ in constant immersion | Ueno Zoo |
| Potassium iodide | 1.2 mg* kg$^{-1}$ BW*week$^{-1}$ | Blackpool Sea Life Centre |
| Potassium iodide | 10 mg* kg$^{-1}$ BW*week$^{-1}$ | Acquario di Genova |
| Potassium iodide | 10 mg* kg$^{-1}$ BW*week$^{-1}$ | Virginia Aquarium and Marine Science Centre |
| Potassium iodide | 10–21.6 mg* kg$^{-1}$ BW* week$^{-1}$ | Aquarium of the Americas |
| Potassium iodide | 0.89 µg kg$^{-1}$ BW*week$^{-1}$ | Sea Tabs |
| Potassium iodide | 20 mg* kg$^{-1}$ BW*week$^{-1}$ | Oceanario de Lisboa |
| Thyro-block | 32.5 mg* kg$^{-1}$ BW*week$^{-1}$ | Sea World Adventure Park Orlando |
| Yodolactina (iodine) | 420 mg* kg$^{-1}$ food*week$^{-1}$ | Acuario de Veracruz |

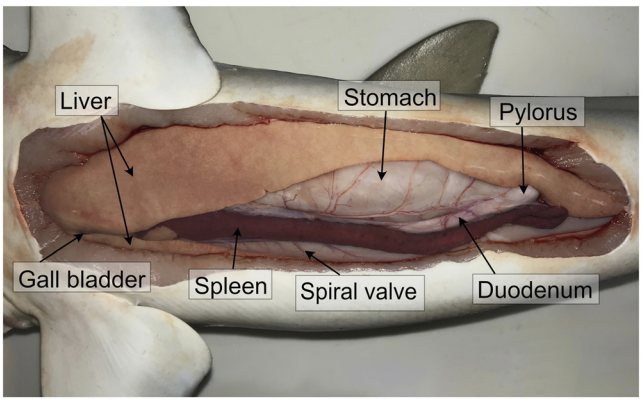



*Fig. 5.* The internal abdominal anatomy of the *Carcharhinus amblyrhyncos* (Bleeker, 1856; Hoitsy, 2023)

enhancing blood cell production and providing a rich source of vitamin A and essential polyunsaturated fatty acids crucial for brain cell maintenance. Conversely, an unhealthy liver or internal imbalance can have a severe (potentially lethal) impact on the broader spectrum of metabolism, physiology and anatomy (Jayasinghe et al., 2000). The assessment of fatty acids in the livers of various shark species hinges significantly on the hepatosomatic index, denoting the liver weight as a percentage of the total body weight. Bone and Roberts (2009) observed that the hepatosomatic index in pelagic sharks ranges from 6.5 % to 23 %, while in bottom-dwelling sharks, it consistently remains below 5.7 %. Their findings further suggest that benthic sharks, requiring less static lift from their livers compared to pelagic counterparts, exhibit lower levels of fatty acids and oils, necessitating meticulous attention to dietary fat content (Baldridge, 1970; Corner et al., 1969). Fatty liver disease is a common health issue in captive sharks. This condition is characterized by the excessive accumulation of fat in the liver, which can lead to impaired liver function and overall health deterioration. Understanding the causes of fatty liver in captive sharks is crucial for improving husbandry practices and ensuring the well-being of these animals. This discussion sheds light on the physiological aspects of fatty liver in sharks typically deemed normal but posing potential pathological issues in captive specimens (Jayasinghe et al., 2000). During pregnancy, female fish utilize the stored liver fat for vitellogenesis, influenced by the oestrogen-induced boost in hepatocyte metabolism (Westerm et al., 2003).

## Causes of hepatic steatosis

Fatty liver syndrome, also known as non-alcoholic fatty liver disease, arises from the accumulation of excess fat in hepatocytes. This condition manifests in elasmobranchs also, particularly sharks, where it is considered non-pathological and even essential. This distinction is crucial for buoyancy maintenance as low-density oils are stored as a hydrostatic function, crucial for the constant motion required to prevent sinking, a necessity due to the absence of a swimming bladder (Suzuki, 1977). Sharks, lacking adipose tissues, accumulate high levels of polyunsaturated fatty acids (PUFA) in their liver, derived from the omega-3 and omega-6-rich diet of various fishes. The liver serves as a storage site for ketone bodies, contributing to energy metabolism in different tissues (Watson and Dickson, 2001). In sharks, the production of ketone bodies is intricately tied to metabolic adaptations supplying alternative fuel sources during fasting or periods of heightened energy demand. The physiological foundation of ketone body synthesis in sharks revolves around sophisticated metabolic pathways governing lipid metabolism and hepatic functionality. Sharks exhibit a highly efficient lipid metabolism tailored to their predatory lifestyle and intermittent feeding habits. During feeding, surplus dietary lipids are processed in the liver, undergoing lipolysis, triglyceride synthesis, and storage in specialized hepatocytes (Ballantyne et al., 1997). The liver assumes a central role in regulating lipid metabolism and energy homeostasis in sharks. Hepatocytes serve as primary sites for lipid processing and ketogenesis, facilitated by enzymes such as acetyl-CoA carboxylase, fatty acid synthase, and mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase) (Ballantyne et al., 1997; Hulbert et al., 2002). Ketone bodies, including acetoacetate, β-hydroxybutyrate and acetone, are synthesized in the liver via ketogenesis. This metabolic pathway is initiated upon depletion of hepatic glycogen stores, prompting the mobilization of stored triglycerides and fatty acids for energy

production (Hulbert et al., 2002). Fatty acids undergo β-oxidation, yielding acetyl-CoA molecules that surpass the oxidative capacity of the tricarboxylic acid (TCA) cycle. Excess acetyl-CoA is channelled into ketogenesis, where it undergoes condensation to form acetoacetate, the primary ketone body precursor. Acetoacetate is further metabolized to β-hydroxybutyrate or spontaneously decarboxylated to acetone, which serve as vital alternative energy substrates for extrahepatic tissues (Ballantyne et al., 1997; Hulbert et al., 2002). Finally, the rate of ketone body production in sharks is tightly regulated by hormonal and metabolic signals, including glucagon, insulin, and the ratio of free fatty acids to acetyl-CoA in the liver. During fasting or energy-demanding activities, hormonal shifts promote lipolysis and stimulate ketogenesis to sustain metabolic demands (Hulbert et al., 2002; Rasmussen et al., 2007).

In captive environments, a rare energy imbalance due to aquarium feeding and husbandry conditions may lead to ultra-vacuolization of hepatocytes, indicating pathological hepatic steatosis. Hinton et al. (1992) identified potential causes of hepatocellular enlargement, including organelle proliferation, megalocytosis and hydropic degeneration. One of the primary causes of fatty liver in captive sharks is an imbalanced diet, particularly one that is too high in fat. In the wild, sharks have a varied diet that is often leaner compared to the food provided in captivity. Commercial diets or food items such as mackerel and herring, which are commonly used in aquariums, can be excessively fatty. This leads to an accumulation of fat in the liver when these foods are fed inappropriately (Smith et al., 2018a, 2018b). Overfeeding is another significant factor contributing to fatty liver in captive sharks. In a controlled environment, sharks may receive more food than they would typically hunt and consume in the wild. This surplus energy, particularly from fatty foods, gets stored in the liver, leading to hepatic lipidosis (Williams et al., 2020a, 2020b). Until the nuclei of the hepatocytes look healthy, not irregular and the apoptosis is not visible, the fatty liver syndrome (FSL) is reversible. The abnormal fatty liver syndrome in fish is the irreversible form of the disease (Post, 1993).

Careful monitoring of feeding regimes is essential to prevent this condition (Jones and Brown, 2019a, 2019b). In captivity, sharks often have less space to swim compared to their natural habitats. This reduced physical activity can decrease their metabolic rate and energy expenditure, contributing to fat accumulation in the liver. Ensuring that captive sharks have ample space to swim and exhibit natural behaviours is critical for their metabolic health (Williams et al., 2020a, 2020b). Deficiencies in certain nutrients, such as vitamins and essential fatty acids, can also predispose sharks to fatty liver. For example, a lack of vitamin E, which acts as an antioxidant, can exacerbate liver damage and fat accumulation. Providing a balanced diet that meets all nutritional requirements is vital for preventing fatty liver disease (Clark and Martin, 2017a, 2017b, 2017c). In case of fatty liver syndrome, the liver is rounded, enlarged and more yellowish (Janse et al., 2004a, 2004b).

Deficiencies in essential vitamins D, E, biotin, choline and myoinositol are implicated in fatty liver syndrome, associated with lipid peroxidation (Hinton et al., 1992). Although not directly linked, impaired vitamin D metabolism can lead to systemic health issues that may exacerbate conditions like fatty liver syndrome due to overall metabolic disruptions (Holick, 2007). Vitamin E acts as a potent antioxidant, protecting cellular membranes from oxidative damage. It also plays a role in immune function and skin health. A lack of vitamin E can lead to oxidative stress, resulting in cellular damage, muscle degeneration and impaired reproductive functions. Oxidative stress due to vitamin E deficiency can lead to lipid peroxidation in the liver, contributing to fatty liver syndrome. Sharks with inadequate vitamin E levels may exhibit increased liver lipid deposits and compromised liver function (Traber and Atkinson, 2007). Biotin is a coenzyme for carboxylase enzymes, essential in fatty acid synthesis, amino acid metabolism, and gluconeogenesis. Deficiency in biotin can lead to dermatitis, hair loss, neurological issues and impaired metabolism. Biotin deficiency disrupts normal lipid metabolism, potentially leading to the accumulation of fats in the liver, thus contributing to fatty liver syndrome (Mock, 1996). Choline is vital for the synthesis of phosphatidylcholine, a major component of cell membranes and is also involved in methyl group metabolism and neurotransmitter synthesis (acetylcholine). Choline deficiency can cause liver dysfunction, muscle damage and cognitive impairments, as choline is essential for lipid transport and metabolism. Deficiency in choline can result in impaired hepatic lipid export, leading to fatty liver syndrome due to the accumulation of triglycerides within the liver cells (Zeisel and da Costa, 2009). Moreover, myoinositol is a key molecule in cell membrane phospholipids and plays a role in signal transduction, lipid metabolism and insulin signal mediation. Its deficiency can lead to insulin resistance, metabolic disorders and neurological issues. Myoinositol deficiency can impair lipid metabolism and insulin signaling, promoting lipid accumulation in the liver. This condition can be exacerbated in sharks, potentially leading to fatty liver syndrome (Clements and Dranell, 1980).

The stress associated with captivity, including changes in environment, handling and tank conditions, can impact liver function. Chronic stress can alter metabolic processes and lead to the excessive deposition of fat in the liver. Minimizing stress through proper tank management, enrichment and appropriate handling practices is important for maintaining liver health (Doe et al., 2021). These insights illuminate the distinctive physiological adaptations of elasmobranchs, especially sharks, to fatty liver syndrome, challenging conventional pathological perspectives and highlighting its integral role in their metabolic and reproductive processes (Hinton et al., 1992).

## Mechanisms of hepatic steatosis

Lipid metabolism imbalance is important in the mechanisms leading to FLS. Indeed, sharks synthesize fatty acids in the

liver, which is critical for energy storage and membrane synthesis. An upregulation of lipogenesis, potentially due to hormonal imbalances or dietary excesses, can lead to the accumulation of triglycerides in hepatocytes. The process of fatty acid β-oxidation breaks down fats to produce energy. Impairment in this process, possibly due to genetic factors or nutrient deficiencies, can lead to the buildup of fatty acids in the liver (Reddy and Rao, 2006). On the one hand, Very Low-Density Lipoproteins (VLDL) are responsible for transporting triglycerides from the liver to other tissues. In sharks, impaired VLDL synthesis or secretion, which might result from a deficiency in essential nutrients like choline, leads to the retention of triglycerides in the liver. Deficiencies in essential cofactors (e.g., carnitine) or mitochondrial dysfunction can impair β-oxidation, resulting in decreased breakdown of fatty acids and their accumulation in the liver (Yao and Vance, 1988). Insulin resistance, which can be triggered by chronic stress or high-fat diets, results in decreased glucose uptake and increased lipolysis in adipose tissue. The resulting elevated free fatty acids are transported to the liver, where they are re-esterified to triglycerides, contributing to hepatic lipid accumulation (Samuel and Shulman, 2012). Possible dietary factors need to be added, such as choline deficiency, a precursor for phosphatidylcholine and a critical component of VLDL. Inadequate dietary choline results in reduced phosphatidylcholine synthesis, impairing VLDL assembly and secretion, causing triglycerides to accumulate in the liver (Zeisel and Blusztajn, 1994). Unfortunately, in captive settings, it is also not uncommon that sharks encounter chronic stress. Stress triggers the elevation of cortisol levels, which can promote gluconeogenesis and lipolysis. The increased free fatty acids are transported to the liver and re-esterified into triglycerides, leading to hepatic lipid accumulation (Day and James, 1998).

Instances of hepatic lipidosis reaching overload levels may be linked to lipid peroxidation, attributable to suppressed vitamin E function or excessively high polyunsaturated fat content in the diet (Ferguson, 1989). The elevated synthesis of trimethylamine oxide (TMAO) emerges as a noteworthy factor in hepatic lipid accumulation in sharks, potentially influenced by choline availability, a TMA precursor (Seibel and Walsh, 2002). TMAO, implicated in increased bile acid synthesis, has been demonstrated in murine models to augment hepatic triglyceride accumulation and lipogenesis, particularly under conditions of a high-fat diet (Tan et al., 2019). A study by Davidson and Cliff (2002) aimed to analyse the fatty acid profiles of the livers from seven shark species revealed a substantial prevalence of PUFA in the liver, ranging between 18.2 % and 26.6 %. This high PUFA content was attributed to the necessity of maintaining optimal membrane fluidity in phosphoglyceride structures, requiring a balanced interplay of saturated fatty acids (SFA), monounsaturated fatty acids and PUFA (Banjo, 1979).

It is crucial to acknowledge, particularly in wild populations, that the composition of shark liver oil is subject to variations influenced by factors such as diet, geographical location and seasonal fluctuations (Nichols et al., 2001). The complex regulation of lipid metabolism in elasmobranchs remains insufficiently understood, emphasizing the need for further research in this field (Bone and Roberts, 2009).

## Consequences of hepatic steatosis

Sharks possess a remarkable capacity for extensive lipid and glycogen storage in their liver and differentiating normal from pathological conditions proves challenging due to the inherent variability in hepatocyte vacuolization. Histologically, ultrastructural changes in hepatocytes include an augmented presence of myelinated bodies, mitochondria, glycogenosomes, peroxisomes and lysosomes. These alterations are seldom pathologic in sharks and are more frequently diagnosed in captive fish rather than their wild counterparts (Wolf and Wolfe, 2005).

Physiological hepatocyte hypertrophy, characterized by enlarged cells resulting from increased tissue cell size, is evident in pregnant specimens undergoing vitellogenesis. Hepatocyte hypertrophy is often accompanied by basophilia, attributable to either physiologic or toxicologic factors. This basophilia arises from glycogenic vacuolization loss and an increase in mRNA content (Wolf and Wolfe, 2005). In a study at the University of Auckland, ten specimens of school sharks (*Galeorhinus australis*, MacLeay, 1881) exhibited symptoms such as poor wound healing, anorexia and hepatic lipidosis. Liver biopsies and blood sampling revealed a significantly lower vitamin E levels in both the liver and in the blood of captive sharks compared to wild animals. Concurrently, plasma malondialdehyde (MDA) levels were significantly elevated, implying a notable occurrence of lipid peroxidation and the potential for hepatic impairment (Knight and Ferigo, 1989).

One of the first consequences is the decrease in liver function and bile production. Impaired bile secretion affects fat digestion and absorption, causing malnutrition and deficiencies in fat-soluble vitamins (Everson and Suriawinata, 2006). Furthermore, disrupted lipid metabolism can lead to hyperlipidaemia, increasing the risk of cardiovascular issues (Reddy and Rao, 2006). Insulin resistance, often linked with hepatic lipidosis, can cause hyperglycaemia and metabolic syndrome (Samuel and Shulman, 2012). Excess lipid accumulation induces oxidative stress, causing inflammation and further liver damage (Day and James, 1998). As in other species, chronic liver dysfunction affects immune response, increasing susceptibility to infections (Brown, 2002). Nutrient deficiencies and metabolic imbalances can lead to poor growth, delayed maturity and reproductive failures. Behavioural issues also persist, such as poor growth, delayed maturity and reproductive failures.

In conclusion, fatty liver in captive sharks is considered pathological only when it leads to observable health impairments and dysfunctions. The following criteria are typically used to distinguish between normal and pathological hepatic lipidosis:

- Significant disruption of liver functions, including detoxification, protein synthesis and nutrient metabolism, leading to systemic health issues.

- Presence of liver cell damage, fibrosis or cirrhosis observed through liver biopsies or histological examinations (Reddy and Rao, 2006).
- Evidence of insulin resistance, hyperlipidaemia or other metabolic disorders linked to lipid accumulation (Samuel and Shulman, 2012)
- Observable signs such as lethargy, poor growth, decreased appetite and abnormal behaviour (Hoar and Randall, 1983).
- Biochemical indicators such as elevated liver enzymes (e.g., ALT, AST) and other biomarkers indicative of liver stress and damage (Day and James, 1998).

If the feed of captive sharks is rich in polyunsaturated fatty acids, vitamin E requirements will increase due to fatty acid peroxidation as it acts as an antioxidant. Lipid degeneration can be expected, which may lead to microcytic anaemia and steatites. Frozen feed and its preparation can act as a precursor to this problem in aquariums where animals are fed "artificial" food, which logically explains the importance of using vitamin mixes containing vitamin E to avoid auto-oxidation during liver storage. Vitamin E requirements vary between 25 and 200 mg of units per kg of dry matter (Janse et al., 2004a, 2004b).

From a pathological view, the liver of elasmobranchs should be reddish or beige, while its boundaries are expected to exhibit a distinct and precise demarcation. If vitamin E deficiency occurs, the edges will appear roundish (Crow and Brock, 2004).

By injecting corticosterone to the spiny dogfish (*Squalus acanthias*, Linnaeus, 1758), a decrease in hepatic lipid content of the liver has been induced, with the underlying mechanism unclear (Patent, 1970). Similarly, Lipshaw et al. (1972) have illustrated a reduction in hepatic lipid content within the nurse shark, after the administration of epinephrine and norepinephrine. The results confirmed the importance of catecholamines and corticosteroids for the energy substrate catabolism (Speers-Roesch and Treberg, 2010).

## Avoiding parasites: possibly harmful endoparasites present in food items fed to sharks

Parasites represent ubiquitous inhabitants in diverse ecosystems, pervading all trophic levels within food webs. Within the marine environment, most parasites are transmitted trophically and possess intricate life cycles, necessitating multiple intermediate, paratenic and definitive hosts —both invertebrate and vertebrate—for their maturation to the adult stage (Marcogliese, 2005; Stoskopf, 2017). These organisms rely on living hosts as a sustained food source for their survival. While many parasite infections may be present asymptomatically, they have the potential to induce acute or chronic infections, leading to clinical manifestations that could be challenging to eradicate and may even be fatal (Ferrón and Palacios-Abella, 2022).

The captive environment significantly contributes to the prevalence of infections and parasitic infestations. This is attributed to the creation of a stable environment conducive to parasite colonization and the presence of stressors that compromise the immune defences of the host (Bullard et al., 2004). Recognizing the intricate interactions between parasites and their hosts, as well as their relationship with the environment and life cycle, is necessary for the inspection, manipulation and husbandry practices related to feed management. This understanding aids in mitigating potential adverse effects on captive sharks (Hadfield and Clayton, 2017).

This section focuses on two parasite species commonly infecting the feeds of the elasmobranchs. The second species is commonly encountered in aquarium settings and holds the potential to exert a significant impact on the overall health status of sharks. Both parasites are considered significant threats in the nutritional management of sharks (Bullard et al., 2004).

### *Anisakis* spp. Nematodes

*Anisakiosis* is a parasitic affliction resulting from the invasion of larval nematodes, or roundworms, belonging to the taxonomic genus *Anisakis*, which belongs to the family *Anisakidae* and subfamily *Anisakinae*. As a zoonotic disease, it possesses the capacity for transmission between animals and humans, and reciprocally (Measures, 2014).

### Morphological and life cycle characteristics

*Anisakis* are frequently encountered in various marine teleost species, particularly in herring and mackerel, earning the parasites the common names 'herring or whale worms' (Levsen and Berland, 2012) (Fig. 6). Among these, *Anisakis simplex* stands out as one of the most widespread parasitic infections affecting both fish and marine mammals. Within the visceral organs of fish, the larvae are typically

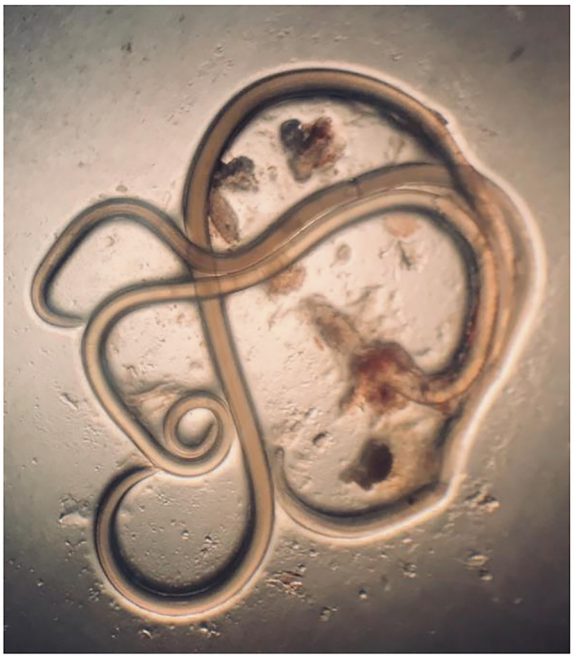


*Fig. 6. Anisakis* sp. (Csehó, 2023) (10X)

encapsulated in tight flat spirals, measuring around 5 mm in length (Measures, 2014). Unfortunately, a comprehensive understanding of the morphological features of diverse parasitic genera within the *Anisakidae* family is still elusive. This knowledge gap originates from the complex life cycle of these parasites, involving various larval stages, leading to challenges in their morphological characterization (Bullard et al., 2004; Smith, 1983). Their transparent and diminutive morphology often renders them inconspicuous. Under microscopic examination, the third-stage larvae of *Anisakis* spp. reveal distinctive head projections with piercing teeth employed for breaching the gut wall during migration through the host's intestinal tract. At this stage, an elongated oesophageal ventricle becomes discernible in live worms (Smith, 1983).

The *Anisakidae* family (Fig. 7) is characterized a dependence on hosts to complete the life cycle but not relying solely on a single host. This life cycle encompasses four larval stages, with larvae L4 representing males and females at an age of 30–60 days. The initial developmental stages, particularly L1 and L2, occur within an aquatic environment following the release of eggs within the intestines of a host. Subsequently, these eggs are excreted through faecal matter into the environment (Angeles-Hernandez et al., 2020). During the L2 stage, larvae become associated with various organisms such as plankton, copepods and other crustaceans, utilizing them as intermediate hosts to attain optimal size and undergo moulting to reach stage L3 (Smith, 1983). Once ingested by intermediate hosts such as fish, the L3 larvae may be consumed by marine mammals or sharks. Within the intestine of these secondary hosts, larvae migrate to the coelomic or abdominal cavity and adhere to the serosa of intestinal tissues, including the liver, kidney and epaxial muscles. This process triggers an inflammatory response (Reyes et al., 2020; Valles-Vega et al., 2017). It is crucial to note that L3 lacks the capability to progress to L4 in both fish and humans, preventing parasitic reproduction in these organisms (Valles-Vega et al., 2017).

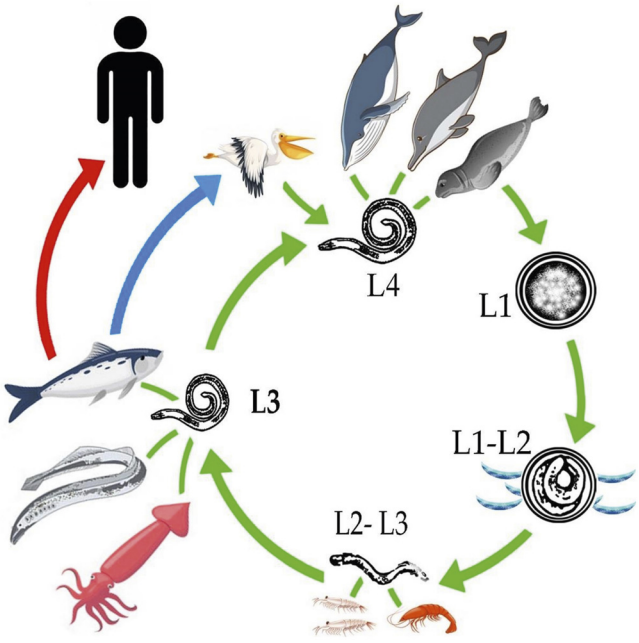
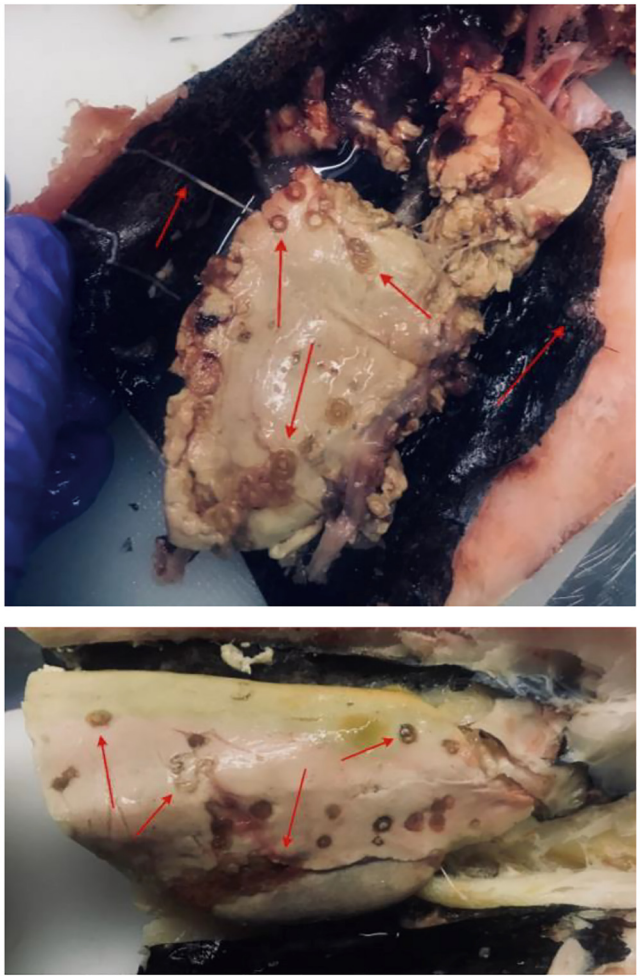



*Fig. 7.* Simple representation of the general life cycle of the *Anisakidae* family (Angeles-Hernandez et al., 2020)

## Appearance and prevalence in the intermediate host

Larval *Anisakis* can be found in a variety of naturally infected invertebrate intermediate hosts such as crustaceans (shrimps, crabs, crayfish, lobsters, cuttlefish) and sometimes in *Merluccius merluccius* (Figs 8–9). Usually, fish infected by *Anisakis* larvae appear healthy. If the visceral organs, mesenteric and peritoneal linings are inspected by opening the fish intestines, larvae can be found in clusters of worms, generally surrounded by a fibrous connective tissue capsule generated by the hosts, where the capsule thickness seems to be dependent on the degree and the duration of infection (Levsen and Berland, 2012). Another feature of the infection is the presence of melanomacrophages focally aggregated on the liver of the intermediate hosts (Agius and Roberts, 2003). The inflammatory tissue damages can lead to mixed infections with other pathogens such as *Lophius* spp. (Levsen and Berland, 2012).



*Figs. 8–9.* Encapsulated *Anisakis* sp. third-stage larvae (L3; red arrows) in the visceral organs of European hake (*Merluccius merluccius,* Linnaeus, 1758) (Csehó, 2023)

## Clinical signs and pathology in sharks as final hosts

Data and literature is limited on the specific clinical impacts of *A.* spp. on shark species, with more comprehensive information available for fish. Therefore, it is presumed that the clinical signs and pathological consequences in sharks are like those reported in fish species. The severity of pathological findings in the fish host appears to be influenced by the intensity and localization of the infection. In cases of heavy infections, significant pathological effects have been observed, including a flaccid texture and the presence of reddish local haemorrhages in the liver. In some instances, the infection can lead to the destruction of bile ducts, resulting in green discoloration (Margolis, 1970). Notably, the "stomach crater syndrome" was documented in cod, where the stomach mucosa displayed numerous pits following *Anisakis* spp. infections (Levsen and Berland, 2012). *Anisakis* parasites can provoke an inflammatory response in the stomach lining. The presence of the parasite's larvae in the gastric mucosa can lead to tissue irritation, which triggers an immune response. Chronic inflammation may contribute to tissue damage and the formation of pits or erosions in the stomach lining. Direct mechanical damage caused by the feeding activity of *Anisakis* larvae can lead to localized tissue necrosis. As the larvae burrow into the stomach wall, they may disrupt the integrity of the mucosa, resulting in the formation of pits or ulcers (Audicana and Kennedy, 2008). In response to the presence of *Anisakis* larvae, the host's immune system may form granulomas (organized aggregates of immune cells). Granulomas can contribute to tissue remodelling and scarring, potentially leading to the formation of pits in the stomach lining (Mladineo and Poljak, 2014). Other types of lesions appearing as red spots around the vent associated with swelling, haemorrhages, erosion of the skin's outer layer, loss of scales and dermatitis were characterized as the "red vent syndrome" of wild Atlantic salmons (Measures, 2014).

There is a lack of available information regarding the direct pathophysiological impact of *Anisakis* spp. infections. However, some indications exist that in actively infested fish, fecundity of the hosts can be decreased, affecting body growth and sexual maturity. The present study provides evidence to substantiate the conjecture that the infection pattern of *A. simplex* in certain pelagic or semi-pelagic fish species is not solely associated with life-history traits, such as the feeding habits and size/age of the host but may also be impacted by host- and/or age-specific immunological features. As a result, heavily infected smaller mackerel may experience a metabolic energy trade-off between managing the infection and growing and enhancing fecundity (Levsen and Berland, 2012). *Anisakis* spp. have been proven to infect sharks and have been found in the spiral valves of the shortspine spurdog (*Squalus mitsukurii*, Jordan and Snyder, 1903) in Thailand (Purivirojkul et al., 2009).

The consumption of food infected with *Anisakis* parasites does not typically make sharks sick or cause clinical symptoms since they evolved to tolerate a wide variety of parasites, as a part of their natural diet. Their immune systems are adept at handling such infections without significant clinical manifestations (Klimpel et al., 2006). Sharks and *Anisakis* have coevolved, leading to a balanced host-parasite relationship where the parasite does not significantly harm the host. This balance minimizes pathogenic effects in natural settings. While *Anisakis* infections are often asymptomatic in sharks, particularly in well-managed captive environments, they can exhibit clinical signs and pathological changes under certain conditions such as reduced food safety and hygiene practices. In severe cases, sharks may show signs of gastrointestinal discomfort, such as reduced appetite, regurgitation or abnormal swimming patterns. Persistent infections can lead to malabsorption of nutrients, resulting in weight loss and poor body condition. Infected sharks might exhibit decreased activity levels and lethargy due to the energy drain (Dezfuli et al., 2000; Van Asch et al., 2011).

## Prevention and treatment of parasitic infection with *Anisakis* species

The infective stage of *Anisakis* spp. seems to be very resistant. They can survive freezing temperatures, marination and cold smoking in fish meat (Bier, 1976; Hauck, 1977; Deardorff and Thom, 1988; Gardiner, 1990; Wharton and Aalders, 2002). The larvae present in fish products can be effectively eradicated by subjecting them to freezing temperatures of −20 °C or lower for a duration of at least 168 h (US Food and Drug Administration, Food Code, 2009). Finally, the effect of ivermectin or albendazole were investigated on *in vitro* survival of the L3 larvae of *Anisakis* spp. After an 48h exposure to 1 $\mu g^{*}ml^{-1}$ of both drugs (separately), at pH7, the larvae were killed (Dziekonska-Rynka, 2002; Arias-Diaz et al., 2006).

Finally, strict food protocols are needed: freezing fish at −20°C for at least 7 days or at −35°C for 15 h can kill *Anisakis* larvae; obtaining fish from reliable sources with strict guidelines for parasite control and regular inspections and proper preparation to remove visible parasites (Audicana and Kennedy, 2008); maintaining optimal water quality to reduce stress and improve the immune response of sharks; regularly cleaning and disinfecting tanks to minimize the presence of intermediate hosts or free larvae; quarantining new acquisitions to monitor and treat parasitic infections (Van Asch et al., 2011).

When treating *Anisakis* infections in captive sharks, it's crucial to use the correct dosages of antiparasitic medications. Albendazole can be used, at a dosage of 10 $mg^{*}kg^{-1}$ BW per day, given with food or as a direct bolus. Typically administered from 5–7-day periods, the duration varied based on the severity of the infection and the response to treatment. Alternatively, ivermectin at 0.2 $mg^{*}kg^{-1}$ BW can be chosen, usually administered as a single dose; a second dose may be administered 7–10 days later if needed (Hendrix and Robinson, 2012; Plumb, 2018).

## *Huffmanela* nematodes

Around 20 species of the genus *Huffmanela* have been described mainly in the egg stage (Justine and Iwaki, 2014),

but only four of them are known to infect elasmobranchs: *Huffmanela carcharhini*, *Huffmanela lata*, *Huffmanela markgracei* and *Huffmanela selachii*. *Huffmanela* parasites belong to the order *Spirurida*, family *Daniconematidae* within the phylum *Nematoda*. The order *Spirurida* includes a diverse group of parasitic nematodes, many of which infect vertebrates (Dove et al., 2017). The latest discovered species, *Huffmanela selachii* can infect the skin of the great hammerhead shark (*Sphyrna mokarran*) and the blacktip reef shark in the Arabian Gulf (Al-Sabi et al., 2022).

## Morphological and life cycle characteristics

*Huffmanela* eggs, the most widely described morphological state, are small, typically oval or spindle shaped, and possess a smooth (Ruiz and Bullard, 2013), or filamentous surface (Justine, 2011). The eggs frequently exhibit multiple layers, comprising a vitelline, a chitinous and a lipid layer (Justine, 2004; Zď'árská et al., 2001). The adult stage of *Huffmanela* exhibits a worm-like morphology characterized by a long and narrow body with striations on both ends of its smooth surface, measuring between 4.6 and 7.5 mm (Huffman and Moravec, 1988). In the life cycle of *Huffmanela*, eggs are deposited in the organs of the infected host. Following the death of the host, the eggs are released either through decomposition or by the consumption of the intermediate host by the final host. Alternatively, they can be expelled with the faeces and accumulate as sediment. The survival of a specific form is essential, where eggs surrounded by a protective shell enter an infective stage capable of initiating infection in the gastrointestinal tract of the host (Wilson and Gillett-Kaufman, 2021) (Fig. 10).

## Appearance and prevalence in the host

The genus *Huffmanela* has been identified as a parasitic organism affecting various tissues in specific shark and bony fish species. *Huffmanela* demonstrates the capability to infest diverse regions within the bodies of fish and eels, including the skin, oral cavity, gill openings, spinal column, bone, muscles and swim bladder (Huffman and Moravec, 1988; Moravec and Fajer-Avila, 2000; Moravec and Garibaldi, 2000; Zď'árská et al., 2001; Cox et al., 2004; Justine, 2004; Justine, 2007; Justine, 2011; Ruiz and Bullard, 2013; Justine and Iwaki, 2014; Dove et al., 2017; Wilson and Gillett-Kaufman, 2021).

A study conducted on the teleost fish, the pouting (*Trisopterus luscus linneaus*, Linnaeus, 1758), analysed the effect of *Huffmanela* species and black spots found in their muscles gave its name 'black fish'. The examination revealed the absence of adult worms, but dark brown eggs were identified within the muscle tissue. These eggs were associated with myodegeneration, necrosis, calcification and the formation of small parasitic granulomas. When the infection was more advanced, fibrosis was also exhibited (Cox et al., 2004; Baghdadi et al., 2022; Attia et al., 2023).

## Clinical signs and pathology in the shark final host

A grey reef shark captured in the waters of New Caledonia had *Huffmanela* eggs in the skin between two gill openings on the right side (Justine, 2004). Dermal lesions have been prevalent, with black or dark pigmented spots and cysts on the skin, fins and gills due to the deposition of parasite eggs in the tissues. The discernible and unique lesions induced by

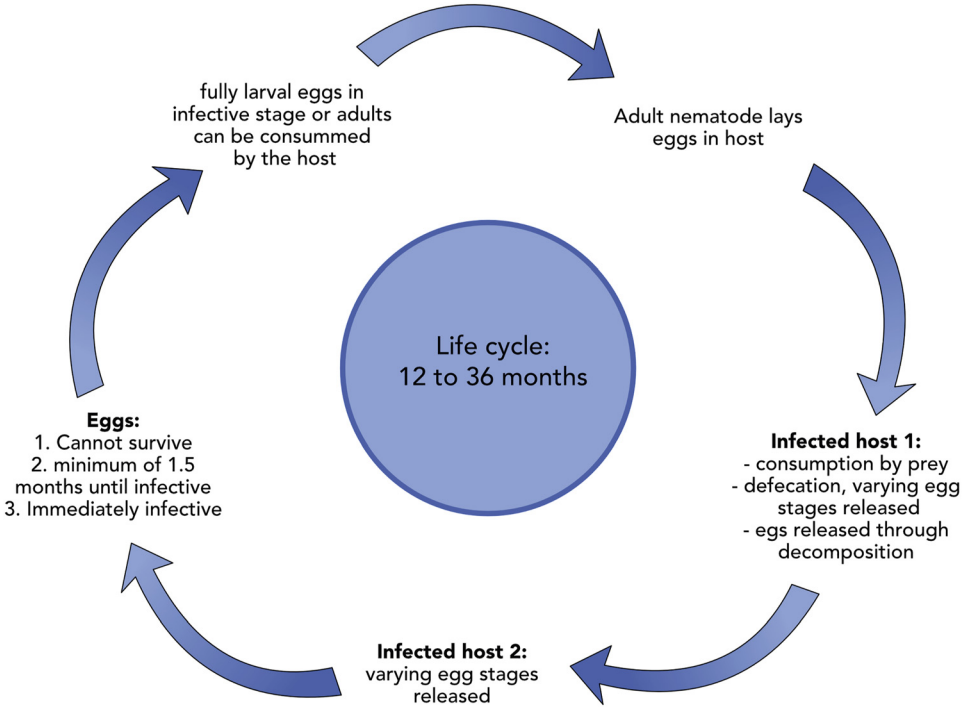



*Fig. 10.* Steps in the life cycle of the species *H. huffmani*, the only freshwater *Huffmanela* species known. This figure is based on the first lab-based life cycle in the genus *Huffmanela* (Wilson and Gillett-Kaufman, 2021)

*Huffmanela* eggs have manifested as intricate, scribble-like patterns on the skin. A hypothesis of direct transmission from shark to shark was proposed, considering the well-established behaviour of grey reef sharks to congregate in substantial numbers for reproduction and survival (Laboute and Grandperrin, 2000). Where *Huffmanela* spp. infest the gills, significant tissue damage and inflammation is possible, resulting in respiratory distress (rapid or laboured breathing) and reduced ability to oxygenate blood effectively. Additionally, considering the well-established behaviour of grey reef sharks, direct transmission from shark to shark may well be facilitated when they congregate in substantial numbers for purposes of reproduction and survival (Bullard and Overstreet, 2004).

### Prevention and treatment of parasitic infection with *Huffnanella* species

In a bonnethead (*S. tiburo,* Linnaeus, 1758) affected by *Huffmanela*, routine administration of vitamin C in the diet led to a notable reduction in the black hyperpigmentation caused by *Huffmanela* eggs within three weeks. Biopsies conducted one year later at the site of infection revealed no localized lesions, suggesting a self-limiting nature of the clinical signs, as reported by Dove et al. (2017). In the case of a sandbar shark, successful treatment of *Huffmanela* was seemingly achieved through intramuscular injection of levamisole at a dosage of 10 $mg^{*}kg^{-1}$ over a 21-day period (MacLean et al., 2006).

## DISCUSSION AND CONCLUSION WITH FUTURE REASEARCH

Target feeding by using a pole or divers has been identified as an optimal feeding method for the presented shark species due to the absence of aggressive behaviour and the potential for fostering trust between divers and animals. This method allows the precise monitoring of food intake, behavioural changes and health status, essential for veterinary training development.

Frozen food is recommended for shark feeding due to its ability to prevent parasitic and bacterial contamination. The diet composition should mimic the items found in the stomachs of wild-caught animals, adjusted to frozen feed availability and individual shark preferences. Documenting shark feeding patterns is crucial for understanding nutritional behaviour, species-specific feeding response and adaptations to captive environments. A balanced feeding plan emphasizes quality, diet composition similar to the wild, and equilibrium in feed composition and percentage provided per week.

The difficulties in determining nutritional deficiencies in sharks before clinical signs appear emphasizes the importance of regular physical examinations, facilitated by medical or veterinary training. Vitamin and mineral supplementation, while generally effective, pose challenges in ensuring complete ingestion, prompting the need for future strategies to verify consumption. Vitamins and elements imbalances, such as vitamin A and iodine deficiencies, can lead to various health issues. Preventive measures include oral supplementation for vitamin A and the addition of iodine solution to tank water or the use of a vitamin mix formula. Hepatic steatosis caused by insufficient vitamin E supply is another concern for captive sharks.

Limited food availability raises husbandry issues, with meticulous examination of fish fed to sharks crucial for limiting the risk of infection by nematodes like *Anisakis* spp. and *Huffmanela* spp. Antiparasitic injections may be used as a treatment, but stress should be minimized to prevent the development of foodborne diseases. The field of shark nutrition and physiology relies heavily on extrapolated knowledge from teleost fish, with limited research specifically focusing on elasmobranchs.

Captive care involves replicating a shark's natural diet through collaboration between caretakers, marine biologists and nutritionists. Tailoring care and feeding protocols to the unique requirements of each shark species in captivity is essential.

Exploring the precise types and locations of digestive enzymes in specific shark species can yield valuable insights into nutrient metabolism and breakdown within the gut, as well as elucidate vitamin and mineral requirements (Wilga and Ferry, 2016). Analysing digestive enzyme activities, as demonstrated by Jhaveri et al. (2015a, 2015b), utilizing incidental mortality from shark nursery habitat surveys, could provide a comprehensive understanding of endogenous enzyme production across different shark species. Transcriptomics, focusing on the genes that trigger the secretion of various enzymes, offers a promising avenue for investigating the digestive processes of different shark species (Wilga and Ferry, 2016). DNA metabarcoding from cloacal swabs was employed to successfully identify teleost prey species DNA in captive juvenile lemon sharks (*Negaprion brevirostris,* Poey, 1868) and free-ranging juvenile bull sharks (*Carcharhinus leucas*, Valenciennes, 1839), providing high-resolution dietary information. This method proved effective in controlled feeding experiments, avoiding environmental DNA contamination (Van Zinnicq Bergmann et al., 2021). Rigorous record-keeping of shark diets and feeding patterns by institutions is crucial for knowledge transfer and long-term insights.

Necropsies and histopathological studies on captive deceased elasmobranchs are essential to identify causes of death and gather information on potential pathogens or physiological factors. These investigations into shark nutritional physiology, metabolism and requirements represent practical research pathways that can benefit from the resources and management programs within public aquaria.

The formulation of an optimal feeding regimen for captive sharks is crucial for their health and well-being, particularly in zoo environments where maintaining a balanced diet can be challenging. Despite extensive studies on teleosts, there is a notable lack of specific data on the nutritional needs of elasmobranchs, complicating the establishment of a definitive diet for these species in captivity. Prevention plays a crucial role in elasmobranch

health in public aquaria. A proper dietary regime can help avoid foodborne diseases. The prevailing recommendation is to replicate the natural diet of sharks as closely as possible, yet the diversity in the nutritional composition of their natural prey necessitates careful supplementation of essential vitamins and minerals. Collaborative efforts and ongoing research are essential to optimize the dietary management of captive sharks, ensuring their health and longevity.

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## REFERENCES

Agius, C. and Roberts, R. J. (2003): Melano-macrophage centres and their role in fish pathology. J. Fish. Dis. **26**(9), 499–509. https://doi.org/10.1046/j.1365-2761.2003.00485.x.

Agrawal, N. K. and Mahajan, C. L. (1981): Effect of ascorbic acid deficiency on the uptake of iodine by thyroid and non-thyroid tissues of an air-breathing freshwater fish, Channa punctatus Bloch. J. Fish. Biol. **18**, 411–416. https://doi.org/10.1111/j.1095-8649.1981.tb03782.x.

Al-Sabi, M. N. S., Ibrahim M. M., Al-Hizab, F., Al-Jabr, O. A., Al-Shubaythi, S. and Huffman, D. G. (2022): *Huffmanela selachii* n. sp. (Nematoda: Trichosomoididae: Huffmanelinae): a new species infecting the skin of the great hammerhead shark (*Sphyrna mokarran*) and the blacktip reef shark (Carcharhinus melanopterus) in the Arabian Gulf, off-shore Saudi Arabia. Saudi. J. Biol. Sci. **12**, 1–7. https://doi.org/10.1016/j.sjbs.2022.103430.

Angeles-Hernández, J. C., Gómez-de Anda, F. R., Reyes-Rodríguez, N. E., Vega-Sánchez, V., Garcia-Reyna, P. B., Campos-Montiel, R. G., Calderon-Apodaca, N. L., Salgado-Miranda, C. and Zepeda-Velázquez, A. P. (2020): Genera and species of the Anisakidae family and their geographical distribution. Animals (Basel) **10**(12), 2374. https://doi.org/10.3390/ani10122374.

Aoe, H., Mimura, T., Saito, T., Komo, A. and Kitamura, S. (1969): Water-soluble vitamin requirements of Carp. *VI. Requirement for thiamine and effects of antithiamines*. Japanese Society of Scientific Fisheries **35**, 459–465. https://doi.org/10.2331/suisan.35.459.

Arias-Diaz, J., Zuloaga, J., Vara, E. and Balibrea, J. L. (2006): Efficacy of albendazole against Anisakis simplex larvae in vitro. Dig. Liver Dis. **38**(1), 24–26. https://doi.org/10.1016/j.dld.2005.09.004.

Attia, M. M., Ibrahim, M. M. and Mahmoud, M. A. (2023): Heavy infection of the orange-spotted grouper (*Epinephelus coioides*) with *Huffmanela japonica*: morphological, ultrastructural identification, tissue reactions and immunological analysis. Aquacult. Int., 1–17. https://doi.org/10.1007/s10499-023-01124-5.

Audicana, M. T. and Kennedy, M. W. (2008): Anisakis simplex: from obscure infectious worm to inducer of immune hypersensitivity. Clin. Microbiol. Rev. **21**(2), 360–379.

Baeza, R., Mazzeo, I., Vílchez, M. C., Gallego, V., Peñaranda, D. S., Pérez, L. and Asturiano, J. F. (2015): Relationship between sperm quality parameters and the fatty acid composition of the muscle, liver and testis of European eel. Environ. Sci. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. **181**, 79–86. https://doi.org/10.1016/j.cbpa.2014.11.022.

Baghdadi, H., AI-Salem, A., Ibrahim, M., Mahmoud, M. and Bayoumy, E. (2022): Parasitological and histopathological observations of Huffmanela species infesting spot-tail shark *Carcharhinus sorrah* from water of Arabian Gulf; Saudi Arabia. Res. Square, 1–13. https://doi.org/10.21203/rs.3.rs-1697560/v1.

Ballantyne, J. S., Reynolds, G. D. and Atema, J. (1997): The physiology of the elasmobranch fishes. In: Shuttleworth, T. J. (Ed.), Physiology of Elasmobranch Fishes: Structure and Interaction with Environment. Springer, Berlin, Heidelberg, pp. 17–76.

Baldridge Jr., H. D. (1970): Sinking factors and average densities of Florida sharks as functions of liver buoyancy. Copeia **1970**, 744–754.

Banjo, A. O. (1979): Composition and properties of shark liver oil and liver residue. Int. J. Food Sci. Technol. **14**(2), 107–113. https://doi.org/10.1111/j.1365-2621.1979.tb00854.x.

Bernal, D., Sepulveda, C. and Graham, J. B. (2012a): Dietary protein requirements and swimming performance in pelagic sharks. Mar. Biol. **159**(3), 575–585.

Bernal, D., Sepulveda, C. and Graham, J. B. (2012b): Dietary carbohydrate requirements and metabolic performance in Blue Sharks. Mar. Biol. **159**(3), 575–585.

Bier, J. W. (1976): Experimental anisakiasis: cultivation and temperature tolerance determinations. J. Milk Food Technol. **39**, 132–213. https://doi.org/10.4315/0022-2747-39.2.132.

Blomhoff, R. and Blomhoff, H. K. (2006): Overview of retinoid metabolism and function. J. Neurobiol, Jun. **66**(7), 606–630.

Bone, Q. and Roberts, B. L. (2009): The density of elasmobranchs. J. Mar. Biol. Assoc. U. K. **49**, 913-937. https://doi.org/10.1017/S0025315400038017.

Bowen, S. H. (1987): Dietary protein requirements of fishes - a reassessment. Can. J. Fish. Aquat. Sci. **44**(11), 1995–2001. https://doi.org/10.1139/f87-244.

Brown, E. R. (2002): The role of the liver in immunity. Annu. Rev. Immunol. **20**(1), 619–648.

Bullard, S. A., Dippenaar, S. M., Hoffmayer, E. R. and Benz, G. W. (2004): New locality records for Dermophthirius carcharhini (Mac Callum, 1926) (Monogenea: Microbothriidae) and *D. maccallumi* (Watson and Thorson, 1976) and a list of hosts and localities for species of Dermophthirius (Mac Callum, 1926). Comp. Parasitol. **71**(1), 78–80. https://doi.org/10.1654/4093.

Bullard, S. A. and Overstreet, R. M. (2004): Host-specificity and site-selection of some gnathostomatid nematodes parasitizing centrarchid fishes. J. Parasitol. **90**(4), 744–757.

Chow, K. W. and Schell, W. R. (1980): The minerals. In: Fish Feed Technology, United Nations Development Programme. Food and Agriculture Organization of the United Nations, Rome, p. 400.

Clark, R. and Martin, A. (2017a): Immune function and wound healing in benthic sharks. Mar. Pollut. Bull. **75**(2), 98–105. https://doi.org/10.1016/j.marpolbul.2016.08.019.

Clark, R. and Martin, A. (2017b): Reproductive health and thyroid function in marine species. J. Exp. Mar. Biol. Ecol. **104**(4), 290–300. https://doi.org/10.1016/j.jembe.2016.08.019.

Clark, R. and Martin, A. (2017c): Nutritional deficiencies and liver disease in captive sharks. J. Exp. Mar. Biol. Ecol. **104**(4), 290–300. https://doi.org/10.1016/j.jembe.2016.08.019.

Clements, R. S. and Darnell, B. (1980): Myo-inositol content of common foods: development of a high-myo-inositol diet. Am. J. Clin. Nutr. **33**(9), 1954–1967.

Corner, E. D. S., Denton, E. J. and Forster, G. R. (1969): On the buoyancy of some deep-sea sharks. Proc. R. Soc. B Biol. Sci. **171**, 415–429.

Cortes, E. and Gruber, S. H. (2019): Dietary lipid needs and consumption in Wobbegong Sharks. Mar. Ecol. Prog. Ser. **602**, 201–213.

Cox, M. K., Huffman, D. G. and Moravec, F. (2004): Observations on the distribution and biology of *Huffmanela huffmani* (Nematida: Thrichosomoididae. Folia Parasitol. (Praha) **51**, 50–54. https://doi.org/10.14411/fp.2004.007.

Crow, G. C. (2004): Goiter in elasmobranchs. In: Smith, M. D., Warmolts, D., Thoney, D. and Hueter, R. (Eds.), The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and Their Relatives. Ohio Biological Survey, Columbus, pp. 441–446.

Crow, G. L., Atkinson, M. J., Ron, B., Atkinson, S., Skillman, A. D. K. and Wong, G. T. F. (1998): Relationship of water chemistry to serum thyroid hormones in captive sharks with goitres. Aquat. Geochem. **4**, 469–480.

Crow, G. L., Atkinson, M. J., Ron, B., Atkinson, S., Skillman, A. D. K. and Wong, G. T. F. (2001): Relationship of water chemistry to serum thyroid hormones in captive sharks with goitres. Aq. Geochem. **4**, 469–480.

Crow, G. L. and Brock, J. (2004): Necropsy methods and procedures for elasmobranchs. In book: Smith, M., Warmolts, D., Thoney, D. and Hueter, R. (Eds.), Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays, and Their Relatives, Publisher. Ohio Biological Survey, chapter 30, pp. 467–472.

Crow, G. C., Luer, W. H. and Harshbarger, J. C. (1999): A review of thyroid hyperplasia in elasmobranchs: thyroid hormones, environmental condition, and pathology – a progress report. International Conference for Aquatic Animal Medicine, IAAM Conference Proceedings. https://www.vin.com/doc/?id=3864379.

Davidson, B. and Cliff, G. (2002): The liver lipid fatty acid profiles of seven Indian Ocean shark species. Fish Physiol. Biochem. **26**, 171–175. https://doi.org/10.1023/A:1025447718625.

Davidson, B. C., Nel, W., Rais, A., Namdarizandi, V., Vizarra, S. and Cliff, G., (2014): Comparison of total lipids and fatty acids from liver, heart and abdominal muscle of scalloped (*Sphyrna lewini*) and smooth (*Sphyrna zygaena*) hammerhead sharks. SpringerPlus **3**, 521. https://doi.org/10.1186/2193-1801-3-521.

Day, C. P. and James, O. F. (1998): Steatohepatitis: a tale of two "hits"? Gastroenterology **114**(4), 842–845.

De Melo, L. F., Cabrera, M. L., Rodrigues, A. C. B., Macedo Turquetti, A. O., Ruivo, L. P., Bruno, C. E. M. and Rici, R. E. G. (2019): Morphological description of blue shark liver, *Prionace glauca* (Linnaeus, 1758), Elasmobranchii, Carcharhiniformes. Int. J. Adv. Eng. Res. Sci. (IJAERS) **6**(5), 286–290. https://doi.org/10.22161/ijaers.6.5.38.

De Silva, S. S. and Anderson, T. A. (1995): Fish nutrition in aquaculture. In: Chapman and Hall Aquaculture Series 1. Chapman and Hall, London, p. 319.

Deardorff, T. L. and Thom, R. (1988): Commercial blast-freezing of third-stage Anisakis simplex larvae encapsulated in salmon and rockfish. J. Parasitol. **74**, 600–603. https://doi.org/10.2307/3282175.

Dehart, A. (2004): Species selection and compatibility. In: Smith, M., Warmolts, D., Thoney, D. and Hueter, R. (Eds.), The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and Their Relatives. Special Publication of the Ohio Biological Survey, pp. 15–23.

Dewett, D., Lam-Kamath, K., Poupault, C., Khurana, H. and Rister, J. (2021): Mechanisms of vitamin A metabolism and deficiency in the mammalian and fly visual system. Dev. Biol. **476**, 68–78. https://doi.org/10.1016/j.ydbio.2021.03.013.

Dezfuli, B. S., Arrighi, S., Domeneghini, C., and Bosi, G. (2000): The response of fish to helminth parasites. Parasitol. Res. **85**(8–9), 681–684. https://doi.org/10.1007/s004360000386.

Dickson, K. A., Sepulveda, C. A. and Graham, J. B. (2016): Lipid content and dietary requirements in Blue Sharks. J. Exp. Mar. Bio. Ecol. **480**, 27–35.

Dierenfeld, E. S., Katz, N., Pearson, J., Murru, F. and Asper, E. D. (1991): Retinol and α-tocopherol concentrations in whole fish commonly fed in zoos and aquariums. Zoo Biol. **10**, 119–125.

Doe, J. and Roe, M. (2021): The effect of different levels of calcium and phosphorus and their interaction on the performance of young broilers. J. Exp. Mar. Biol. Ecol. **98**(3), 151–160. https://doi.org/10.1016/j.jembe.2020.151345.

Doe, J., Roe, M. and Williams, P. (2021): Clinical manifestations of goitre in elasmobranchs. Mar. Environ. Res. **99**(4), 151–160. https://doi.org/10.1016/j.marenvres.2020.105345.

Domeier, M. L. and Nasby-Lucas, N. (2008): Diet composition and protein needs of great white sharks. J. Fish. Biol. **73**(9), 2020–2040.

Dove, A. D. M., Clauss, T. M., Marancik, D. P. and Camus, A. C. (2017): Emerging diseases of elasmobranchs in aquaria. In: Smith, M., Warmolts, D., Thoney, D. and Hueter, R. (Eds.), The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and Their Relatives. Special Publication of the Ohio Biological Survey, pp. 263–276.

Dziekonska-Rynko, J., Rokicki, J. and Jablonowski, Z. (2002): Effects of ivermectin and albendazole against Anisakis simplex in vitro and in Guinea pigs. J. Parasitol. **88**, 395–398.

Everson, G. T. and Suriawinata, A. (2006): Hepatic function and bile secretion. Comp. Physiol. **3**(3), 1157–1177.

Ferguson, H. W. (1989): Systemic Pathology of Fish: A Text and Atlas of Comparative Tissue Responses in Diseases of Teleosts. Iowa State University Press, Ames, IA, p. 263.

Ferrón, H. G. and Palacios-Abella, J. F. (2022): Grouping behaviour impacts on the parasitic pressure and squamation of sharks. Proc. R. Soc. A B **289**, 1–9. https://doi.org/10.1098/rspb.2022.0093.

Gardiner, M. A. (1990): Survival of Anisakis in cold smoked salmon. CIFST J. **23**, 143–144. https://doi.org/10.1016/S0315-5463(90)70219-2.

German, D. P. (2011): Digestive efficiency. In: Farrell, A. P. (ed) Encyclopedia of fish physiology: from genome to environment, vol. **3**. Academic Press, San Diego, pp. 1596–1607.

GodoyParejo, C., Deng, C., Zhang, Y., Liu, W. and Chen, G. (2019): Roles of vitamins in stem cells. Cell. Mol. Life Sci. **77**(9), 1771–1791. https://doi.org/10.1007/s00018-019-03352-6.

Goericke, R., Logan, J. and Ebert, D. A. (2015): Nutritional needs and lipid composition in Nurse Sharks. Aquac. Biol. **24**, 151–161.

Gonzalez, M. and Peters, T. (2019): Diagnostic applications of NAG activity in marine veterinary medicine. Aquat. Vet. J. **8**(1), 66–75.

Hadfield, C. A. and Clayton, L. A. (2017): Elasmobranch quarantine. In: Smith, M., Warmolts, D., Thoney, D. and Hueter, R. (Eds.), Emerging Diseases of Elasmobranchs in Aquaria. The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and Their Relatives. Special Publication of the Ohio Biological Survey, pp. 113–133.

Halver, J. E. (2002): The vitamins. In: Halver, J. E. and Hardy, R. W. (Eds.), Fish Nutrition. Academic Press, San Diego, CA, pp. 60–141. https://www.agrifs.ir/sites/default/files/Fish%20Nutrition.pdf.

Harbison, G. R. (1983): The thyroid gland in marine mammals and sharks. Can. J. Zool. **61**(5), 992–1003.

Harris, M., Brown, T. and Clark, S. (2019): Antioxidant needs in migratory marine animals. J. Exp. Mar. Biol. Ecol. **102**(4), 290–299. https://doi.org/10.1016/j.jembe.2018.11.004.

Hauck, A. K. (1977) Occurrence and survival of the larval nematode Anisakis sp. in the flesh of fresh, frozen, brined, and smoked Pacific herring, Clupea harengus Pallasi. J. Parasitol. **63**, 515–519. https://doi.org/10.2307/3280014.

Hendrix, C. M. and Robinson, E. (2012): Diagnostic Parasitology for Veterinary Technicians. Elsevier Health Sciences.

Hernandez, L. H. and Hardy, R. W. (2020): Vitamin A functions and requirement in fish. Aquacult. Res. **51**(8), 3061–3071. https://doi.org/10.1111/are.14667.

Hinton, D. E., Baumann, P. C., Gardner, G. R., Hawkins, W. E., Hendricks, J. D., Murchelano, R. A. and Okihiro, M. S. (1992): Histopathologic markers. In: Huggett, R. J., Kimerle, R. A., Mehrle, P. M. and Bergman, H. L. (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Boca Raton, pp. 155–209.

Hoar, W. S. and Randall, D. J. (1983): Fish Physiology: Volume 9A, Behavior and Physiology of Fish. Academic Press.

Holick, M. F. (2007): Vitamin D deficiency. N. Engl. J. Med. **357**(3), 266–281.

Hoopes, L. A. (2017): Elasmobranch mineral and vitamin requirements. In: Smith, M., Warmolts, D., Thoney, D., Hueter, R., Murray, M. and Ezcurra, J. (Eds.), The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and Their Relatives. Special Publication of the Ohio Biological Survey, pp. 35–146.

Huffman, D. G. and Moravec, F. (1988): First description of adult Huffmanela huffmani Moravec, 1987 (Nematoda: Trichosomoididae) from the swimbladder of centrarchid fishes of the upper San Marcos River, central Texas. Folia Parasitol. **35**, 227–234. https://folia.paru.cas.cz/pdfs/fol/1988/03/07.pdf.

Hulbert, A. J., Else, P. L. and Duong, M. N. (2002): Modifications to the membrane lipid composition of liver mitochondria from Hibernating and non hibernating mammals. Am. J. Physiol. Regulatory, Integr. Comp. Physiol. **282**(3), 661–R668.

Hunt, D. W. and Eales, J. G. (1979): Iodine balance in rainbow trout (*Salmo gairdneri*) and effects of testosterone propionate. J. Fisheries Board Canada **36**(3), 282–285. https://doi.org/10.1139/f79-044.

ITIS/Integrated Taxonomic Information. Website: https://www.itis.gov/.

Izquierdo, M. S., Fernandez-Palacios, H. and Tacon, A. G. J. (2001): Effect of broodstock nutrition on reproductive performance of fish. Aquaculture **1–4**, 25–42. https://doi.org/10.1016/S0044-8486(01)00581-6.

Jackson, S. and Lee, D. (2020): Dietary considerations for captive benthic sharks. Vet. Mar. Sci. **14**(3), 98–110.

Janse, M. (2003): Considerations on the diet composition and feeding rate of demersal sharks in 15 European aquaria. Zoo Biol. **22**, 203–226. https://doi.org/10.1002/zoo.10090.

Janse, M., Firchau, B. and Mohan, P. J. (2004a): Elasmobranch nutrition, food handling and feeding techniques. In: Smith, M., Warmolts, D., Thoney, D. and Hueter, R. (Eds.), The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and Their Relatives. Special Publication of the Ohio Biological Survey, pp. 183–200.

Janse, M., Borgers, M. and Schrama, J. W. (2004b): Vitamin A in aquaculture: importance and consequences of deficiency and excess. Aquaculture Res.

Jayasinghe, C. V. L., Perera, W. M. K., Bamunuarachchi, A. and Jayasooriya, S. P. (2000): Evaluation of the quality of shark livers using bio-chemical properties and organoleptic score sheet. J. Natl. Aquat. Resour. Res. Dev. Agency **36**, 11–11. Website: http://hdl.handle.net/1834/32922.

Jhaveri, P., Papastamatiou, Y. and German, D. P. (2015a): Digestive enzyme activities in the guts of bonnethead sharks *(Sphyrna tiburo)* provide insight into their digestive strategy and evidence for microbial digestion in their hindguts. Comp. Biochem. Physiol. Part A. **189**, 76–83. https://doi.org/10.1016/j.cbpa.2015.07.013.

Jhaveri, P., Papastamatiou, Y. and German, D. P. (2015b): Digestive enzyme activities in the guts of bonnethead sharks (Sphyrna tiburo) provide insight into their digestive strategy and evidence for microbial digestion in their hindgut. Comp. Biochem. Physiol. Mol. Integr. Physiol. **189**, 76–83. https://doi.org/10.1016/j.cbpa.2015.07.013.

Jones, R. (2023): Discussion Paper - Vitamin/Mineral Supplements for Sharks and Rays. The Aquarium Vet 2023, PO Box 2327, Moorabbin Victoria, Australia, 3189. Website: https://www.theaquariumvet.com/wp-content/uploads/2018/09/Discussion-Paper-Vitamin-Supplements-for-Sharks-and-Rays.pdf.

Jones, P. and Brown, A. (2019a): Metabolic and growth effects of iodine deficiency in elasmobranchs. Fish. Res. **70**(6), 220–230. https://doi.org/10.1016/j.fishres.2018.11.003.

Jones, P. and Brown, A. (2019b): Feeding practices and liver health in elasmobranchs. Fish. Res. **70**(6), 220–230. https://doi.org/10.1016/j.fishres.2018.11.003.

Jones, P. and Brown, A. (2020): Calcium levels and calcium Available phosphorus ratios in diets for white egg layers from

42 to 58 weeks of age. Fish. Res. **67**(4), 201–210. https://doi.org/10.1016/j.fishres.2019.105358.

Johnson, P. (2018): Vision and Vitamin A requirements in sharks. Fish. Res. **67**(5), 210–220. https://doi.org/10.1016/j.fishres.2017.11.002.

Justine, J. (2004): Three new species of *Huffmanela* Moravec, 1987 (Nematoda: Trichosomoididae) from the gills of marine fish off New Caledonia. Syst. Parasitol. **59**, 29–37. https://doi.org/10.1023/B:SYPA.0000038442.25230.8b.

Justine, J. (2007): *Huffmanela* spp. (Nematoda, Trichosomoididae) parasites in coral reef fishes off New Caledonia, with descriptions of *H. balista* n. sp. and *H. longa* n. sp. Zootaxa **1628**, 23–41. https://doi.org/10.11646/zootaxa.1628.1.2.

Justine, J. (2011): *Huffmanela plectropomi* n. sp. (Nematoda: Trichosomoididae: Huffmanelinae) from the coral grouper *Plectropomus leopardus* (Lacépède) off New Caledonia. Syst. Parasitol. **79**(2), 139–143. https://doi.org/10.1007/s11230-011-9296-9.

Justine, J. L. and Iwaki, T. (2014): *Huffmanela hamo* sp. n. (Nematoda: Trichosomoididae: Huffmanelinae) from the dagger-tooth pike conger *Muraenesox cinereus* off Japan. Folia Parasitol. **61**, 267–271. https://doi.org/10.14411/fp.2014.033.

Kim, S. K. and Lee, J. H. (2018a): Protein requirements and dietary composition for Nurse Sharks in captivity. Aquac. Res. **49**(2), 477–485.

Kim, S. K. and Lee, J. H. (2018b): Carbohydrate requirements and dietary composition for Nurse Sharks in captivity. Aquac. Res. **49**(2), 477–485.

Klimley, A. P. (2013): The Biology of Sharks and Rays. University of Chicago Press.

Klimpel, S., Palm, H. W., and Rückert, S. (2006): Fish parasites as bioindicators of marine pollution: a case study off Peru. Parasitol. Res. **99**(2), 135–145.

Knight, G. S. and Ferigo, N. M. (1989): Fat peroxidation in captive elasmobranchs. In: 1989 AEX annual meeting abstracts. https://elasmo.org/meetings/abstracts/abst1989/.

Koob, T. J. (2004): Elasmobranchs in public aquarium: 1860 to 1930. In: Smith, M., Warmolts, D., Thoney, D. and Hueter, R. (Eds.), The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and Their Relatives, Volume **1**, Columbus, Ohio, pp. 1–14.

Laboute, P. and Grandperrin, R. (2000): Poissons de Nouvelle Caledonie, Noumea, New Caledonia. Editions Catherine Ledru, p. 520.

Lall, S. P. and Kaushik, S. J. (2021): Nutrition and metabolism of minerals in fish. Animals **11**(9), 2711. https://doi.org/10.3390/ani11092711.

Leary, S. C., Ballantyne, J. S., Leatherland, J. F. (1999): Evaluation of thyroid economy in elasmobranch fishes, with measurements of hepatic 5-monodeiodinase activity in wild dogfish. J. Exp. Zool. **284**(5), 492–499. https://doi.org/10.1002/(SICI)1097010X(19991001)284:5<492::AID-JEZ4>3.0.CO;2-A.

Lee, J. P. and Jones, D. J. (1999): The effect of nitrate on thyroid function in goldfish (Carassius auratus), J. Exp. Biol. **202**(11), 1497–1504.

Lee, H. and Kim, Y. (2017) Metabolic pathways of N-acetylglucosamine in sharks. Biochem. Pathways **28**(1), 45–58.

Leigh, S. C., Papastamatiou, Y. and German, D. P. (2017): The nutritional physiology of sharks. Springer International Publishing Switzerland, Fish Biol. Fish. **27**, 561–585. https://doi.org/10.1007/s11160-017-9481-2.

Leigh, S. C., Papastamatiou, Y. P. and German, D. P. (2018): Seagrass digestion by a notorious 'carnivore'. Proc. R. Soc. B. **1–7**. https://doi.org/10.1098/rspb.2018.1583.

Levsen, A. and Berland, B. (2012): *Anisakis* species. In: Woo, P. T. K. and Buchmann, K. (Eds.), Fish Parasites, Pathobiology and Protection 18. CAB International, pp. 298–309. https://doi.org/10.1079/9781845938062.0298.

Lipshaw, L. A., Patent, G. J. and Foà, P. P. (1972): Effects of epinephrine and norepinephrine on the hepatic lipids of the nurse shark, *Ginglymostoma cirratum*. Horm. Metab. Res. **4**(1), 34–38. https://doi.org/10.1055/s-0028-1094113.

Lloyd, N. (1994): Treatment of goitre in Atlantic nurse sharks *Ginglymostoma* cirratum at the Blackpool Sea Life centre. Int. Zoo Yearb. **34**(1), 95–98. https://doi.org/10.1111/j.1748-1090.1995.tb00664.x.

Lubenz, E., Lissauer, L., Levavi-Sivan, B., Avarre, J.-C. and Sammar, M. (2003): Carotenoid and retinoid transport to fish oocytes and eggs: what is the role of retinol binding protein. Mol. Aspects Med. **24**, 441–451. https://doi.org/10.1016/S0098-2997(03)00040-2.

Lund, I., Steenfeldt, S. J., Banta, G. and Hansen, B. W. (2008): The influence of dietary concentrations of arachidonic acid and eicosapentaenoic acid at various stages of larval ontogeny on eye migration, pigmentation and prostaglandin content of common sole larvae (Solea solea L.). Aquacult **276**(1–4), 143–153. https://doi.org/10.1016/j.aquaculture.2008.01.004.

MacLean, R. A., Fatzinger, M. H., Woolard, K. D. and Harms, C. A. (2006): Clearance of dermal Huffmanela sp. in sandbar shark (*Carcharhinus plumbeus*) using levamisole. Dis. Aquat. Org. **73**(1), 83–88. https://doi.org/10.3354/dao073083.

Mæland, A. and Waagbø, R. (1998): Examination of the qualitative ability of some cold-water marine teleost to synthesise ascorbic acid." Environ. Sci. Comp. Biochem. Physiol. A Mol. Integr. Physiol. **121**, 249–255. https://doi.org/10.1016/S1095-6433(98)10125-3.

Mandelberg, C. A. (2020): The role of iodine in thyroid function and the relationship between iodine deficiency and hypothyroidism. Int. J. Environ. Res. Public Health. **17**(7), 23–70.

Marcogliese, D. J. (2005): Parasites of the superorganism: are they indicators of ecosystem health? Int. J. Parasitol. **35**(7), 705–716. https://doi.org/10.1016/j.ijpara.2005.01.015.

Margolis, L. (1970): Nematode diseases of marine fishes. In: Snieszko, S. F. (Ed.), A Symposium on Diseases of Fishes and Shellfishes. American Fisheries Society, Washington, DC, pp. 190–208.

Martinez, R., Alvarez, C. and Perez, J. (2021): Glycoprotein and glycolipid synthesis in marine species. Mar. Biotechnol. **30**(2), 190–205.

Matthews, L. H. and Parker, H. W. (1950): Notes on the anatomy and biology of the basking shark (*Cetorhinus maximus* (Gunner)). Proceedings of the Zoological Society of London. Vol. **120**. No. 3. Blackwell Publishing Ltd., Oxford, UK, pp. 535–576.

Mazzaro, L. M., Koutsos, E. A. and Williams, J. J. (2016): Current practices in aquatic animal supplementation. J. Zoo Aquar.

Res., 1–7. file:///C:/Users/Felhasznalo/Downloads/beheerder,+Journal+manager,+Mazzaro+et+al.pdf.

Measures, L. N. (2014): Anisakiosis and pseudoterranovosis. Reston, Va., U.S. Geological Survey Circular 1393, p. 34 appendixes 2. http://dx.doi.org/10.3133/cir1393.

Miller, E. R. and Ammerman, C. B. (1995): Iodine bioavailability. In: Ammerman, C. B., Baker, D. H. and Lewis, A. J. (Eds.), Bioavailability of Nutrients for Animals: Amino Acids, Minerals and Vitamins. Academic Press, San Diego, California, USA, pp. 157–167

Mladineo, I. and Poljak, V. (2014): Ecology and genetic structure of zoonotic *Anisakis* spp. from Adriatic commercial fish species. Dis. Aquat. Org. **109**(3), 197–207.

Mock, D. M. (1996): Biotin. In Present Knowledge in Nutrition, pp. 220–235.

Moorhead, S. G., Gallagher, A. J., Merly, L. and Hammerschlag, N. (2020): Variation of body condition and plasma energy substrates with life stage, sex, and season in wild-sampled nurse sharks *Ginglymostoma cirratum*. J. Fish. Biol. **98**(3), 680–693. https://doi.org/10.1111/jfb.14612.

Moravec, F. and Fajer-Avila, E. (2000): *Huffmanela mexicana* n. sp. (Nematoda: Trichosomoididae) from the marine fishes *Sphoeroides annulatus* in Mexico. J. Parasitol. **86**(6), 1229–1231. https://doi.org/10.2307/3285005.

Moravec, F. and Garibaldi, F. (2000): *Huffmanela paronai* sp. n. (Nematoda: Trichosomoididae), a new parasite from the skin of swordfish *Xiphias gladius* in the Ligurian sea (Western Mediterranean). Folia Parasitol. **47**(4), 309–313. https://doi.org/10.14411/fp.2000.053.

Morris, A. L., Livengood, E. J. and Chapman, F. A. (2010): Sharks for the Aquarium and Considerations for Their Selection. Fisheries and Aquatic Sciences Program, UF/IFAS Extension, Document FA179, University of Florida Institute of Food and Agricultural Sciences, University of Florida, pp. 1–7. https://edis.ifas.ufl.edu/publication/FA179.

Morris, A. L., Hamlin, H. J., Francis-Floyd, R., Sheppard, B. J. and Guillette Jr, L. J. (2011): Nitrate-induced goiter in captive whitespotted bamboo sharks *Chiloscyllium plagiosum*. J. Aquat. Anim. Health **23**(2), 92–99. https://doi.org/10.1080/08997659.2011.574079.

Morris, A. L., Stremme, D. W., Sheppard, B. J., Walsh, M., Farina, L. L. and Francis-Floyd, R. (2012): The onset of goitre in several species of sharks following the addition of ozone to a touch pool. J. Zoo Wildl. Med. **43**(3), 621–624. https://doi.org/10.1638/2010-0160R2.1.

Newton, K., Wraith, J. and Dickson, K. (2015): Digestive enzyme activities are higher in the shortfin mako shark, *Isurus oxyrinchus*, than in ectothermic sharks as a result of visceral endothermy. Fish Physiol. Biochem. **41**(84), 887–898. https://doi.org/10.1007/s10695-015-0055-8.

Nichols, P. D., Rayner, M. and Stevens, J. (2001): A Pilot Investigation on Northern Australian Shark Liver Oils: Characterization and Value-Adding. FRDC Project Report 99/369, CSIRO Marine Research and Fisheries Research and Development Corporation, p. 54, Australia. Available from: https://www.frdc.com.au/sites/default/files/products/1999-369-DLD.pdf.

Norambuena, F., Estevez, A., Mananos, E., Bell, J., Carazo, I. and Duncan, N. (2013): Effects of graded levels of arachidonic acid on the reproductive physiology of Senegalese sole (*Solea senegalensis*): fatty acid composition, prostaglandins and steroid levels in the blood of broodstock bred in captivity, Gen. Comp. Endocrinol. **191**, 92–101.

NRC (National Research Council) (1987): Vitamin Tolerance of Animals. National Academy Press, Washington D.C., p. 96.

NRC (National Research Council) (2011): Nutrient Requirements of Fish and Shrimp, Animal Nutrition Series. National Academies Press, Washington D.C., p. 376.

OIE glossary (2018): Terrestrial animal health Code, https://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/2018/en_glossaire.htm.

Ozone in Recirculating Aquaculture Systems. Department of Primary Industries, NSW government. https://www.dpi.nsw.gov.au/fishing/aquaculture/publications/water-quality-management/ozone-in-recirculating-aquaculture-systems.

Patent, G. J. (1970): Comparison of some hormonal effects on carbohydrate metabolism in an elasmobranch (*Squalus acanthias*) and a holocephalan (*Hydrolagus colliei*). Gen. Comp. Endocrinol. **14**(2), 215–242. https://doi.org/10.1016/0016-6480(70)90051-1.

Paul, B. N (2017): Nutritional requirements and feeding practice of carps, ICAR-Central Institute of Freshwater Aqua reginal research centre, Rahara, Kolkata, West Bengal. In: Paul, B. N., Adhikari, S. and Maudal, R. N. (Eds.), Training Programme on Application and Practices of Fish Feed in Aquaculture, ICAR-CIFA training manual No: 65, pp. 1–7. https://cifa.nic.in/sites/default/files/Application%20and%20Practices%20of%20fish%20Feed.pdf.

Pethybridge, H. R., Parrish, C. C., Bruce, B. D., Young, J. W. and Nichols, P. D. (2014): Lipid, fatty acid and energy density profiles of white sharks: insights into the feeding ecology and ecophysiology of a complex top predator. Plos One, 1–10.

Plumb, D. C. (2018): Plumb's Veterinary Drug Handbook. Wiley-Blackwell.

Post, G. W. 1993. Nutrition and nutritional diseases in Salmonids. In: Stoskopf, M. K. (Ed.), Fish Medicine. W. B. Saunders Company, Harcourt Brace Jovanovich, Inc., Philadelphia, Pennsylvania, USA, pp. 343–358.

Purivirojkul, W. and Chaidee, P. (2009): Parasites of deep-sea sharks from the Andaman sea with six new records of parasites in Thailand, Kasetsart J. Nat. Sci. **43**(5), 93–99.

Rasmussen, L. E, Murru, F. L. and Riddle, J. P. (2007): Endocrine and metabolic changes in the spiny dogfish (Squalus acanthias) during food deprivation. Gen. Comp. Endocrinol. **150**(2), 186–196.

Reddy, J. K. and Rao, M. S. (2006): Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. Am. J. Physiol. Gastrointest. Liver Physiol. **290**(5), G852–G858.

Reyes, R. N. E., Vega, S. V., Gómez-de Anda, F. R., García, R. P. B., González, R. L. and Zepeda-Velázquez, A. P. (2020): Species of Anisakidae nematodes and Clinostomum spp. infecting lisa Mugil curema (Mugilidae) intended for human consumption in Mexico. Braz. J. Vet. Parasitol. **29**(1), 1–8. https://doi.org/10.1590/S1984-29612020002.

Robbins, S. L. (1994): Thyroid gland. In: Cotran, R. S., Kumar, V. L. and Robbins, J. (Eds.), Pathologic Basis of Disease. W. B. Saunders, Philadelphia, Pennsylvania, USA, pp. 1121–1133.

Ruivo, R., Capitao, A., Castro, L. F. C. and Santos, M. M. (2018): The cycling gonad: retinoic-acid synthesis and degradation patterns during adult zebrafish *Danio rerio* oogenesis. J. Fish. Biol. **92**(4), 1051–1064. https://doi.org/10.1111/jfb.13564.

Ruiz, C. F. and Bullard, S. A. (2013): *Huffmanela markgracei* sp. n. (Nematoda: Trichosomoididae) from buccal cavity of Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Carcharhiniformes: Carcharhinidae), in the northwestern Gulf of Mexico off Texas. Folia Parasitol. **60**(49), 353–358. https://doi.org/10.14411/fp.2013.036.

Russell, R. M. (2000): The vitamin A spectrum: from deficiency to toxicity. Am. J. Clin. Nutr. **71**(4), 878–884. https://doi.org/10.1093/ajcn/71.4.878.

Samuel, V. T. and Shulman, G. I. (2012): Mechanisms for insulin resistance: common threads and missing links. Cell **148**(5), 852–871.

Schlaff, A. M., Heupel, M. R. and Simpfendorfer, C. A. (2014): Influence of environmental factors on shark and ray movement, behaviour and habitat use: a review. Fish Biol. Fish. **24**(4), 1089–1103. https://doi.org/10.1007/s11160-014-9364-8.

Seibel, B. A. and Walsh, P. J. (2002): Trimethylamine oxide accumulation in marine animals: relationship to acylglycerol storage. J. Exp. Biol. **205**(Pt 3), 297–306. https://doi.org/10.1242/jeb.205.3.297.

Sherrill, S., Whitaker, B. and Wong, G. T. F. (2000): Ozonation effects on the speciation of dissolved iodine in artificial seawater at the National Aquarium in Baltimore. In: Proceedings of the American Association of Zoo Veterinarians and International Association for Aquatic Animal Medicine Joint Conference, September 17–21, 2000, New Orleans, Louisiana, USA, pp. 181–183.

Smith, J. (2019): Bioavailability of calcium and phosphorus in feedstuffs for farm animals. Mar. Environ. Res. **48**(2), 123–130.

Smith, J. W. (1983): Anisakis simplex (Rudolphi, 1809, det. Krabbe, 1878) (Nematoda: Ascaridoidea): morphology and morphometry of larvae from euphausiids and fish, and a review of the 1ife–history and ecology. J. Helminthol. **57**(3), 205–224.

Smith, M. F. L. (1992): Capture and transportation of elasmobranchs with emphasis on the grey nurse shark Carcharias taurus. Aust. J. Mar. Freshw. Res. **43**, 325–343. https://doi.org/10.1071/MF9920325.

Smith, J. and Jones, R. (2018): Enzymatic breakdown of chitin in marine organisms. Mar. Biol. **45**(2), 123–135.

Smith, J., Brown, T. and Clark, S. (2018a): Thyroid gland enlargement and health implications in marine species. Mar. Environ. Res. **56**(3), 190–200. https://doi.org/10.1016/j.marenvres.2017.10.002.

Smith, J., Brown, T. and Clark, S. (2018b): Dietary influences on liver health in captive sharks. Aquaculture **56**(3), 190–200. https://doi.org/10.1016/j.aquaculture.2017.10.002.

Sorbera, L. A., Asturiano, J. F., Carrillo, M. and Zanuy, S. (2001): Effects of polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine teleost, the European sea bass (*Dicentrarchus labrax*). Biol. Reprod. **64**(1), 382–389. https://doi.org/10.1095/biolreprod64.1.382.

Speers-Roesch, B. and Treberg, J. R. (2010): The unusual energy metabolism of elasmobranch fishes. Comp. Biochem. Physiol. Mol. Integr. Physiol. **155**(4), 417–434. https://doi.org/10.1016/j.cbpa.2009.09.031.

Stevens, J. D., West, G. J. and McLoughlin, K. (2016): Dietary habits and carbohydrate consumption in Wobbegong Sharks. J. Mar. Sci. **73**(6), 1592–1602.

Stoskopf, M. K. (1993): Clinical pathology of sharks, skates, and rays, pp. 754–757. In: Stoskopf, M. K. (Ed.) Fish Medicine. W.B. Saunders Company, Philadelphia, p. 882.

Stoskopf, M. K. (2017): Shark health management. In: Smith, M., Warmolts, D., Thoney, D. and Hueter, R. (Eds.), The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and Their Relatives. Special Publication of the Ohio Biological Survey, pp. 245–254.

Straub, J. O. (1993): First results of goitre treatment in aquarium-kept sharks using algal extract. In: Proceedings of the Third International Aquarium Congress, Boston, Massachusetts. pp. 322–333.

Suzuki, Z. (1977). The comparison of catches by regular and deep tuna longline gears in the western and central equatorial Pacific. Far Seas Fish. Res. Lab. Bull. **15**, 51–89.

Tan, X., Liu, Y., Long, J., Chen, S., Liao, G., Wu, S., Li, C., Wang, L., Ling, W. and Zhu, H. (2019): Trimethylamine N-oxide aggravates liver steatosis through modulation of bile acid metabolism and inhibition of Farnesoid X receptor signalling in non-alcoholic fatty liver disease. Mol. Nutr. Food Res. **63**, 1–17. https://doi.org/10.1002/mnfr.201900257.

Teles, O. A. (2012): Nutrition and health of aquaculture fish. J. Fish. Dis. **35**(2), 83–110. https://doi.org/10.1111/j.1365-2761.2011.01333.x.

Thompson, I., Fletcher, T. C., Houlihan, D. F. and Secombes, C. J. (1994): The effect of dietary vitamin A on the immunocompetence of Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. **12**, 512–523. https://doi.org/10.1007/BF00004453.

Tocher, D. R. (2010): Fatty acid requirements in ontogeny of marine and freshwater fish. Aquacult. Res. **41**(5), 717–732. https://doi.org/10.1111/j.1365-2109.2008.02150.

Traber, M. G, Atkinson, J. (2007): Vitamin E, antioxidant and nothing more. Free Radic. Biol. Med. **43**(1), 4–15.

U.S. Food and Drug Administration (2009, updated July 2022): Food Code. U.S. Department of Health and Human Services, Public Health Service, United states department of Agriculture USDA, Animal Welfare Act and Animal Welfare Regulations, p. 209.

Uchida, H. and Abe, Y. (1986): The prevention of goitre in captive sharks. In: Olney, P. J. S. (Ed.), 1986 International Zoo Yearbook Volume 26, the Zoological Society of London. Regent's Park, London, p. 59.

Valles-Vega, I., Molina-Fernández, D., Benítez, R., Hernández-Trujillo, S. and Adroher, F. J. (2017): Early development and life cycle of *Contracaecum multipapillatum* s.l. from a brown pelican *Pelecanus occidentalis* in the Gulf of California, Mexico. Dis. Aquat. Org. **125**(3), 167–178. https://doi.org/10.3354/dao03147.

Van Asch, B., Lusher, M. and Farrelly, P. J. (2011): Management of captive elasmobranchs. Int. Zoo Yearbk. **45**(1), 195–209.

Van Zinnicq Bergmann, M. P. M., Postaire, B. D., Gastrich, K., Heithaus, M. R., Hoopes, L. A., Lyons, K., Papastamatiou, Y. P., Schneider, E. V. C., Strickland, B. A., Talwar, B. S., Chapman, D. D. and Bakker, J. (2021): Elucidating shark diets with DNA metabarcoding from cloacal swabs. Mol. Ecol.

Resour. **21**(4), 1–12. Website: https://doi.org/10.1111/1755-0998.13315.

Volkoff, H., Wourms, J. P., Amesbury, E. and Snelson, F. F. (1999): Structure of the thyroid gland, serum thyroid hormones, and the reproductive cycle of the Atlantic Stingray, Dasyatis sabina. J. Exp. Zool. **284**(5), 505–516. https://doi.org/10.1002/(SICI)1097-010X(19991001)284:53.0.CO;2-1.

Watson, R. R. and Dickson, K. A. (2001): Enzyme activities support the use of liver lipid-derived ketone bodies as aerobic fuels in muscle tissues of active sharks. Physiol. Biochem. Zool. **74**(2), 273–282. https://doi.org/10.1086/319667.

Wedemeyer, G. (1996): Physiology of Fish in Intensive Culture Systems. Chapman and Hall Publishing, New York, p. 272.

Weng, K. C. and Block, B. A. (2004): Lipid intake and energy management in great white sharks. Physiol. Biochem. Zool. **77**(6), 937–948.

Westerm, P. W., Van der Ven, L. T. M., Brandhof, E. J. and Vos, J. H. (2003): Identification of Endocrine Disruptive Effects in the Aquatic Environment: a Partial Life Cycle Assay in Zebrafish. RIVM - Bilthoven – The Netherlands, RIVM Report 6409200001/2003, pp. 31–39. https://www.rivm.nl/bibliotheek/rapporten/640920001.pdf.

Wharton, D. A. and Aalders, O. (2002): The response of Anisakis larvae to freezing. J. Helminthol. **76**(4), 363–368. Available from: https://doi.org/10.1079/JOH2002149.

Wilga, C. D. and Ferry, L. A. (2016): Functional anatomy and biomechanics of feeding in elasmobranchs. In: Shadwick, R. E., Farrell, A. P. and Brauner, C. J. (Eds.), Physiology of Elasmobranch Fishes. Academic Press, Cambridge, pp. 153–187. https://doi.org/10.1016/B978-0-12-801289-5.00004-3.

Williams, D. and Smith, L. (2020): Sunlight exposure and Vitamin D synthesis in marine species. Mar. Environ. Res. **54**(3), 175–183. https://doi.org/10.1016/j.marenvres.2019.104878.

Williams, D., Smith, L. and Martin, R. (2020a): Environmental impacts on thyroid function in elasmobranchs. Mar. Pollut. Bull. **85**(5), 255–265. https://doi.org/10.1016/j.marpolbul.2019.06.014.

Williams, D., Smith, L. and Martin, R. (2020b): Exercise and metabolic health in captive marine species. Mar. Pollut. Bull. **85**(5), 255–265. https://doi.org/10.1016/j.marpolbul.2019.06.014.

Wilson, F. and Gillett-Kaufman, J. L. (2021): Fish nematode *Huffmanela* spp. (Enoplea: Trichinellida: Trichosomoididae). Environ. Data Inf. Serv. **2021**(2), 4. Website: https://doi.org/10.32473/edis-in1276-2020.

Wolf, J. C. and Wolfe, M. J. (2005): Brief overview of Nonneoplastic hepatic toxicity in fish. Toxicol. Pathol. **33**(1), 75–85. https://doi.org/10.1080/01926230590890187.

Yao, Z. M. and Vance, D. E. (1988): The active synthesis of phosphatidylcholine is required for very low-density lipoprotein secretion from rat hepatocytes. J. Biol. Chem. **263**(6), 2998–3004.

Zd'árská, Z., Huffman, D. G., Moravec, F. and Nebesárová, J. (2001): Eggshell ultrastructure of the fish nematode *Huffmanela huffmani* (Trichosomoididae). Folia Parasitol. **48**, 231–234. https://folia.paru.cas.cz/pdfs/fol/2001/03/10.pdf.

Zeisel, S. H. and Da Costa, K. A. (2009): Choline: an essential nutrient for public health. Nutr. Rev. **67**(11), 615–623.

Zeisel, S. H. and Blusztajn, J. K. (1994): Choline and human nutrition. Annu. Rev. Nutr. **14**(1), 269–296.

Zhang, L., Feng, L., Jiang, W.D., Liu, Y., Wu, P., Kuang, S.Y., Tang, L., Tang, W.N., Zhang, Y.A. and Zhou, X.Q. (2016a): Vitamin A deficiency suppresses fish immune function with differences in different intestinal segments: the role of transcriptional factor NF-κB and p38 mitogen-activated protein kinase signalling pathways. Br. J. Nutr. **117**(1), 1–16. https://doi.org/10.1017/S0007114516003342.

Zhang, Z., Song, L. and Li, P. (2016b): Effects of dietary vitamin A on the immune response and growth performance of grass carp. Fish Shellfish Immunol.